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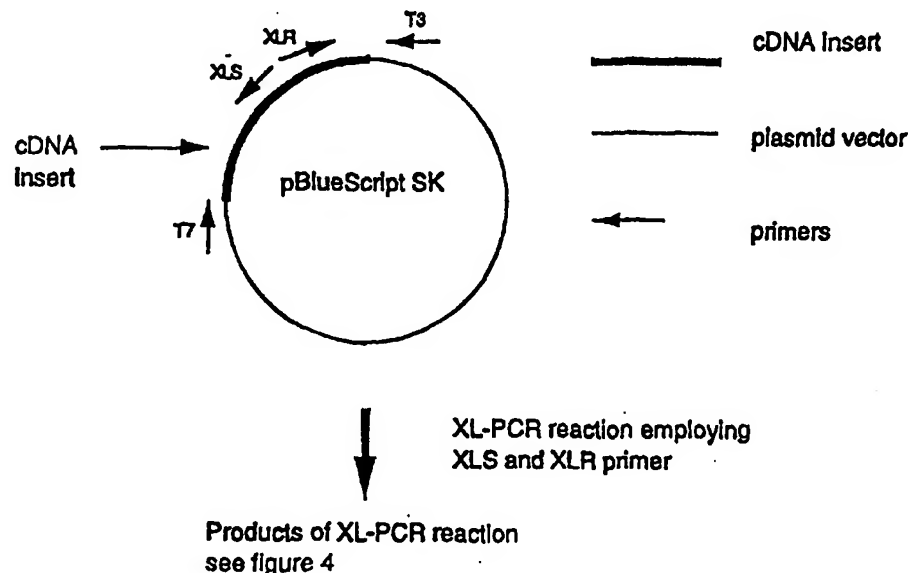
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(54) Title: IMPROVED METHOD FOR OBTAINING FULL-LENGTH cDNA SEQUENCES



(57) Abstract

A method for obtaining longer cDNA sequences is provided. The method utilizes a known genomic DNA sequence or a partial cDNA sequence, such as can be obtained from GenBank partial cDNAs. Two PCR primers are designed to correspond to the ends of the known partial sequence and to anneal to DNA in a cDNA library so as to initiate extension away from the known cDNA and the other primer. The primers are added to a cDNA library with appropriate enzymes and extend through additional DNA sequence to produce PCR products, which are subsequently purified and sequenced to provide new sequences. The new sequences are then compared with the known partial cDNA sequence for areas of overlap, and the sequence is extended beyond the overlapping areas to provide longer DNA sequence.

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IMPROVED METHOD FOR OBTAINING FULL-LENGTH cDNA SEQUENCES**TECHNICAL FIELD**

The present invention is in the field of molecular biology
5 and more particularly, in the field of recombinant DNA technology.

BACKGROUND ART

PCR has become a widely used nucleic acid amplification
technique since it was first presented by Kary Mullis at the Cold
10 Spring Harbor Symposium (Mullis K et al (1986) Cold Spring Harbor
Symp Quant Biol 51: 263-273). PCR requires that a pair of primers
be generated from known sequences. However, in many cases,
sequence is available only from one end of a DNA segment. Several
methods have been developed to sequence an entire gene once a
15 partial nucleotide sequence is available. As more partial cDNA
sequences become available in the world's genetic databanks, more
efficient and economical methods will be sought for then obtaining
the complete gene.

PCR has become a widely used technique to complete genes for
20 which a partial sequence is already known. Gene-specific primers
and primers located in the vector into which the cDNAs have been
cloned are used for this purpose. However, this method is limited
by the use of primers complementary to vector sequence which is
common to all clones in the library. This results in an abundance
25 of non-specific PCR-products which have to be cloned and
sequenced. Multiple rounds of amplifications with nested primers
might be required. These additional operations increase the
incorporation of errors.

Gobinda, Turner and Bolander (1993) in PCR Methods and
30 Applications 2:318-22 disclose "restriction-site PCR" as a direct
method of retrieving unknown sequence which is adjacent to a known
locus by using universal primers. First, genomic DNA is amplified
in the presence of restriction site oligonucleotides and a primer

specific to the known region. Next, those products are subjected to a second round of PCR with the same restriction site oligonucleotides and another specific primer internal to the first one. Subsequently, the products of the last round of PCR are
5 transcribed with an appropriate RNA polymerase and sequenced with a reverse transcriptase and an end-labeled specific primer internal to the second specific PCR primer. Gobinda et al. present data concerning Factor IX for which they identified a conserved stretch of 20 nucleotides in the 3' noncoding region of
10 the gene.

Inverse PCR is the first method that reported successful acquisition of unknown sequences starting with primers based on a known region (Triglia T, Peterson MG, and Kemp DJ (1988) *Nucleic Acids Res.* 16:8186). Inverse PCR employs a strategy in which
15 several restriction enzymes are used to generate a suitable fragment in the known region. The segment is then circularized by intramolecular ligation and used as a PCR template with divergent primers created from the known region. However, the requirement of multiple restriction enzyme digestions followed by multiple
20 ligations (even before PCR is started) make the procedure slow and expensive (Gobinda et al. *Supra*).

Capture PCR, first disclosed by Lagerstrom M, Parik J, Malmgren H, Stewart J, Patterson U and Landegren U (1991) *PCR Methods Applic.* 1:111-19, is a method for PCR amplification of DNA
25 fragments adjacent to a known sequence in human and YAC DNA. As noted by Gobinda et al. *supra*, that method also requires multiple restriction enzyme digestions and ligation of an engineered double-stranded primer before PCR. Although the restriction and ligation reactions are carried out simultaneously in this method,
30 the requirement of extension reaction, immobilization of the extended product, two rounds of PCR and purification of template prior to sequencing render it cumbersome and time consuming as well.

Walking PCR, disclosed by Parker JD, Rabinovitch PS, and Burmer GC (1991) Nucleic Acids Res 19:3055-60, teaches a method for targeted gene walking via PCR. Although this method also permits retrieval of unknown sequence, Gobinda et al, *supra*, note that it requires oligomer-extension assay followed by identification and gel purification of the desired band prior to sequencing. Such extra steps again limit the applicability of the method.

The enzymes originally used in PCR were limited in their ability to reliably amplify long pieces of nucleic acids over 3kb. One of the explanations for this limitation seems to be the misincorporation of nucleotides resulting in non-basepairing mismatches which these enzymes often fail to extend.

Only the mixture of two enzymes, rTth DNA-Polymerase and Vent, the latter of which has so-called "proofreading" activity, and the optimization of amplification conditions finally overcame this limitation and made amplification of pieces of DNA of up to 40kb possible.

The most common way to identify genes expressed in a certain tissue at a certain time is the isolation of the mRNA of that particular tissue and the conversion of this mRNA into so-called cDNA (complementary DNA). This cDNAs are subsequently cloned into a vector (plasmid or Lambda) and amplified by transfection into E.coli cells resulting in a so-called cDNA library.

First and most important to researchers attempting to obtain a complete gene is that the enzymes used in converting mRNA into cDNA are limited in their ability to produce complete copies of the existing mRNAs. This requires the researcher to isolate multiple cDNA clones of the gene of interest using specific probes and analyze each of these isolates for a complete cDNA of the gene of interest. This process is called screening of cDNA libraries.

A major problem facing molecular biologists is finding the most efficient method to use to obtain a full-length cDNA from a

partial sequence. Such sequences are appearing with increasing frequency in GenBank, from commercial cDNA libraries and privately prepared libraries. The inventive method disclosed herein is a contribution to that art.

5

DISCLOSURE OF THE INVENTION

An improved method for extending the DNA sequence of a known fragment of DNA sequence is provided. The method may be used for extending known DNA sequences of genomic or cDNA origin. The method utilizes the polymerase chain reaction (PCR) and includes the steps of:

10

a) combining a first and second PCR primer with nucleic acid from a cDNA library, or pools of cDNA libraries, expected to contain said partial cDNA, or said partial cDNA that has been extended, or a genomic library, under conditions suitable for synthesis of nucleic acid PCR products from the first and second primers, wherein said first and second primers are capable of annealing to opposite strands of the partial cDNA or genomic DNA and initiating nucleic acid synthesis in an outward manner and wherein the first primer is capable of being extended by DNA polymerase in an antisense direction and the second primer is capable of being extended in a sense direction,

15

20

b) purifying the PCR products, and

c) identifying extended nucleotide sequences derived from said partial cDNA or said genomic DNA. In one embodiment of the present invention, the method of identifying the extended nucleotide sequences comprises nucleic acid sequencing. In another embodiment of the present invention, the method proceeds with repeating steps 6a through 6c on the nucleotide sequences identified in step 6c.

25

30

In another embodiment of the present invention, there is a method for extending the nucleotide sequence of a partial complementary DNA (cDNA) using polymerase chain reaction (PCR), comprising the steps of a) combining a first and second PCR primer

with nucleic acid from a cDNA library, or pools of cDNA libraries, expected to contain said partial cDNA, or said partial cDNA that has been extended, or a genomic DNA library, under conditions suitable for synthesis of nucleic acid PCR products from the first and second primers, wherein said first and second primers are capable of annealing to opposite strands of the partial cDNA and initiating nucleic acid synthesis in an outward manner and wherein the first primer is capable of being extended by DNA polymerase in an antisense direction and the second primer is capable of being extended in a sense direction,

- b) purifying the PCR products,
- c) ligating the purified PCR products under conditions suitable for the formation of circular, closed nucleic acid,
- d) transforming a host cell with the circular, closed nucleic acid and culturing the transformed host cell under conditions suitable for growth,
- e) recovering said circular closed nucleic acid from the cultured, transformed host cell, and
- f) identifying extended nucleotide sequences derived from said partial cDNA or said genomic DNA.

The present invention also provides a method for extending known genomic DNA sequences which may be used for the detection and amplification of 5' untranslated nucleotide sequences and/or promoter sequences.

Also provided is an isolated DNA molecule comprising SEQ ID NO:11, the DNA for a novel human purinergic P2U receptor.

Also provided is an isolated DNA molecule comprising SEQ ID NO:12, the DNA for a novel human C5a-like seven transmembrane receptor.

These and other objects, advantages and features of the present invention will become apparent to those persons skilled in the art upon reading the details of the structure, synthesis, formulation and usage as more fully set forth below, reference

being made to the accompanying figures forming a part hereof.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 is a flow chart of the steps in the inventive method.

5 Figure 2 shows a typical plasmid obtained from the excision process of a lambdaZAP cDNA library. Typically 250-300 base pairs of the sequence are obtained in the high-throughput sequence operation. The clone is partially sequenced from the 5' end with T3 as a sequencing primer.

10 Figure 3 is a representation of the next step, in which pBLUESCRIPT SK plasmids in a cDNA library are used as a template and the two specially designed primers (XLR and XLS) amplify plasmids containing the gene of interest. Only plasmids containing priming sites for both XL-PCR primers and the gene of
15 interest will be amplified during the XL-PCR reaction.

Figure 4 is a representation of the amplified DNA segments which have been obtained through the XL-PCR reaction and consequently purified after separating the products on an agarose gel. For best results, the cDNA library used as a template should
20 be synthesized by random priming to assure the availability in this step of different amplified length of DNA (3' end) between the XLS priming site and the T7 priming site in the vector. The length of the 5' end (between the XLR priming site and the T3 priming site) in the vector will vary in size depending on how
25 much of the mRNA of the gene of interest had been converted into cDNA during the cDNA library synthesis.

Figure 5 shows how the purified DNA segments containing the plasmid and the gene of interest are religated to form a circular plasmid and transformed into bacteria for amplification. Here
30 chemically competent E. coli cells were transformed and grown on petri dishes containing LB agar and 25 mg/L carbenicillin (2XCarb) for antibiotic selection.

Figure 6 shows schematically how pure samples of clones were

obtained from the different E. coli colonies grown in the procedure shown in Figure 5 (also Step 1 purification, Step 2 religation and Step 3 transformation in Figure 6). These clones are screened in Step 4 for additional sequence of the gene of interest at the 5' end. For this purpose the clones were analyzed by a PCR reaction employing the XLR primer and the T3 vector primer. The size of the resulting product will indicate how much additional sequence upstream of the XLR priming site each clone contains.

Figures 7A through 7H show the results of the inventive method, in which a partial sequence from Incyte clone 14770, which was similar to heat shock protein 90, was successively sequenced to obtain a full-length cDNA.

Figures 8A through 8F show the results of the inventive method, in which a partial sequence from Incyte clone 87058 which was similar to cathepsin was successively sequenced to obtain extensions of the cDNA.

MODES FOR CARRYING OUT THE INVENTION

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which this invention belongs. All patents and publications referred to herein are incorporated by reference herein.

Before the present compounds, variants, formulations and methods for making and using such are described, it is to be understood that this invention is not limited to the particular compounds, variants, formulations or methods described, as such enzymes, formulations and methodologies may, of course, vary. The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting since the scope of protection will be limited only by the appended claims.

In the specification and appended claims, the singular forms

"a", "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a high-fidelity PCR enzyme" includes mixtures of such enzymes and any other enzymes fitting the stated criteria, reference to the method includes reference to one or more methods for obtaining full-length cDNA sequences which will be known to those skilled in the art or will become known to them upon reading this specification.

The present method provides a way to utilize a genomic DNA library or a plasmid cDNA library (either obtained by cloning cDNAs directly into a plasmid vector or by converting a Lambda library into a plasmid library by known methods e.g. Lambda ZAP excision or Lambda ZIPLOCK conversion) which has been used for sequencing cDNAs, as a source to obtain much longer DNAs and in certain cases complete genes of partially known DNA sequences. The steps disclosed herein are based on cDNA libraries but equally apply to genomic DNA libraries.

This new method utilizes PCR kits which enable the researcher to amplify long pieces of DNA. The XL-PCR amplification kit (Perkin-Elmer) was employed. However, equivalent products may be available from other major suppliers. This novel method allows one person to process multiple genes (up to 96 genes) at a time and obtain extended or complete sequence (possibly full-length) of the cDNAs of interest within 6-10 days. This compares very favorably with current competitive methods like screening with labelled probes which allow one worker to process only about 3-5 genes and obtain initial results in 14-40 days. This represents an increase in throughput of at least 1000%.

This increased efficiency is possible because of the inventive combination of steps shown in the flow chart (Figure 1). First, primer design and synthesis (based on a known partial sequence) can be performed in about two days. The PCR amplification can be performed in 6-8 hours. Multiple libraries

can be pooled and therefore screened at the same time. The next steps of purification and ligation take about one day. Then transformation and growing up the bacteria take one day. Then screening for clones with additional sequence of the genes of interest by PCR takes approximately five hours. The next steps of DNA preparation and sequencing of the selected clones can be performed in about one day. This totals 6-7 days. At the end of this time, one has usually obtained a much longer cDNA sequence, assuming such a longer cDNA existed in the libraries than what was initially sequenced. If the new sequence is a complete gene, then the goal has been reached. If the complete sequence has not been obtained, one still has a much longer sequence than before, and this longer sequence can be used to design primers to repeat the procedure on the same or another library. The choice of library is up to the researcher, but a preferred library is one that has been size-selected to include only larger cDNAs.

This method presumes that one already has partial cDNA sequences, either from a publicly available database or the scientist's own earlier research, including but not limited to earlier preparation of a cDNA library whose cDNAs have been partially sequenced. The cDNA library may have been prepared with oligo dT or random primers. The difference between oligo dT and randomly primed libraries is that a randomly primed library will have more sequences which contain 5' ends of cDNAs. A randomly primed library may be particularly useful for further work when the oligo dT library does not yield a complete gene. Random priming of the library also helps yield more cDNA sequences of different lengths. Library preparation techniques which promote longer insert sizes will in turn permit the sequencing of more complete cDNAs. Obviously, the larger the protein, the less likely it is that the complete cDNA will be found in a single plasmid.

Figure 2 shows a typical plasmid containing a cDNA which had

been partially sequenced from the 5' end with T3 as a primer. The top darkened portion represents the insert containing the gene of interest.

Step 1: PCR-amplification of cDNA-clones containing the gene of interest

5 The first step of this method requires the design of two primers based on the known sequence. The known sequence can be obtained by those skilled in the art either by a wet lab method or from the many publicly available DNA databases. One primer is
10 synthesized to be extended in an antisense direction (XLR) and the other in the sense direction (XLS or XLF). In effect, the primers are designed to anneal to either end of the known sequence and to be extended "outward" from there to generate amplicons containing new, unknown sequences of the genes of interest. This is
15 different from typical PCR, in which the primers are designed to amplify a known sequence in a direction "inward" toward each other.

The primers need to be designed in a way displaying optimal criteria for extra long PCR. A program like Oligo 4.0s (National
20 Biosciences, Inc., Plymouth MN) can be employed for this purpose. In general primers should be 22-30 nucleotides in length, consist of a GC content of 50% or more and anneal at 68°C-72°C to the target. Hairpin structures and primer-primer dimerizations must be avoided.

25 Primers varying from the conditions described above may result in amplification of the desired targets providing extension conditions have been adjusted.

Figure 3 shows the next step, in which a cDNA library is used as a template and the two primers (XLR and XLS) amplify plasmids
30 containing the gene of interest. In this step, it is very helpful to use PCR enzymes which provide high fidelity and copy long sequences, such as that provided in the XL-PCR kit (Part No. N808-0182, Perkin Elmer, Applied Biosystems, Foster City, CA).

Generally, kit instructions should be followed, including suggestions to optimize concentrations of various reagents. In the examples disclosed *infra*, 25pMol of each primer worked well. Template (plasmid library) concentrations can be varied (see Examples *infra* for details). It is essential to thoroughly resuspend the enzyme in solution prior to use, especially if the solution has been stored at -20°C. If the enzyme is not adequately resuspended, its effectiveness is impaired. The preferred system is setup initially in two layers, employing Ampliwax* PCR Gems. However, efficiency can be increased by avoiding the use of these Gems and initiating amplification by using the "hot-start" technique by adding Magnesium, which is essential for amplification, at 82° C.

Although various cycling conditions are detailed in the examples *infra*, the following cycling conditions have been found to be optimal with the MJ PCT200 thermocycler (MJ Research, Watertown, MA). Times and temperatures may be varied to optimize conditions in different thermocyclers.

Step 1	94° for 60 sec (initial denaturation)
Step 2	94° for 15 sec
Step 3	65° for 1 min
Step 4	68° for 7 min
Step 5	Repeat step 2-4 for 15 additional times
Step 6	94° for 15 sec
Step 7	65° for 1 min
Step 8	68° for 7 min + 15 sec/cycle
Step 9	Repeat step 6-8 for 11 additional times
Step 10	72° for 8 min
Step 11	4° for 0.00 sec (to hold at 4°)

At the end of these 28 cycles, 50 µl of the reaction mix is removed; on the remaining reaction mix, an additional 10 additional cycles are run, as outlined below:

Step 1	94° for 15 sec
Step 2	65° for 1 min
Step 3	68° for (10 min + 15 sec)/cycle
Step 4	Repeat step 1-3 for 9 additional times
Step 5	72° for 10 min

Next a 5-10 μ l aliquot of the reaction mixture can be analyzed on a mini-gel to determine which reactions were successful.

Step 2: Purification of amplicons containing the gene of interest

5 Figure 4 is a graphical representation of the amplified cDNA segments which have been separated on an agarose gel. Note that there are a variety of lengths of cDNA. Although the rest of the method could be performed using all extended cDNA species, the method can proceed optionally after selecting the largest products
10 (likeliest to provide the remainder of the full-length gene). Some of the larger species may in fact be hybrid clones which contain two cDNA inserts as a result of malfunction during the cDNA library construction which may represent an incomplete digestion with the restriction enzyme at the end of the cDNA
15 synthesis. Such amplified hybrid clones, also called chimera, could result in overlooking the correct targeted extensions.

Successful reaction products should be purified on an agarose gel (preferentially low agarose concentrations 0.6-0.8% should be used) or other appropriate method. An appropriate volume of
20 reaction mixture should be loaded to obtain good separation of the products and to separate them from the plasmid library (template) still in the reaction mixture. Contamination with the template cDNA library will result in transformants which don't contain the desired gene and will require an extensive screening of many
25 colonies. The bands representing the genes of interest are then cut out of the gel and purified using a method like the QIAQuick gel extraction kit (Qiagen, Inc., Chatsworth, CA).

Step 3: Cloning of amplicons containing the gene of interest

30 Eventual overhangs are converted into blunt ends to facilitate religation and cloning of the products. For this purpose, Klenow enzyme (3 units/reaction mixture) and dNTP's (0.2 mM final concentration) are added and the reaction is incubated at room temperature for 30 min. The Klenow enzyme is then

inactivated by incubating the reaction at 75° for 15 min.

The products are then ethanol precipitated and redissolved in 13 µl of ligation buffer containing 1 mM ATP. 1ml T4-DNA ligase (15 units) and T4 Polynucleotide kinase (5 units) are added and
5 the reaction is incubated at room temperature for 2-3 hours or overnight at 16°C.

3µl of the ligation mixture are transformed into 40ml of competent E.coli cells (prepared with a standard protocol). 80µl of SOC medium are added and after 1 hour of recovery of the cells
10 at 37°C the whole transformation mixture is plated on LB-agar 2XCarb-containing petri plates.

Step 4: Screening of cloned products

The next day 8 or 12 colonies are randomly picked from each plate and grown in individual wells of a sterile 96-well
15 microtiter plate (e.g. 96 Well Cell Culture Cluster, Catalog No. 3799, Costar Corp., Cambridge, MA 02140). Each well contains 150µl of LB/2XCarb medium. Thus, each row of the microtiter plate contains twelve clones from the same extension reaction. The cells are grown over night at 37°C.

20 The next day, 5 µl of these overnight cultures are transferred into a non-sterile 96-well plate (Falcon 3911 Microtest III™, Flexible Assay Plate, Becton Dickinson, Oxnard, CA) and diluted 1:10 with water. 5µl of each dilution are then transferred into a PCR array (e.g., Cycleplate, Robbins Scientific Corp., Sunnyvale,
25 CA). To obtain a 1X final concentration of PCR reagents, 15 µl of a 1.33X concentrated PCR mix are added to each well. Another way of efficient screening for extension products is the multiplex PCR method where multiple specific primers are pooled and submitted to the same reaction, therefore increasing the efficiency of setting
30 up the screening mixtures. Addition of the PCR-template (individual cultures) has been improved by the use of a 96-pin tool with which an aliquot of all 96 cultures grown as described

above can be transferred into the PCR-screening mix in a matter of 1-2 minutes.

For PCR amplification, the final concentrations are 1X for PCR mix, 5 μ M of each of a vector primer and one or both of the gene specific primers used for the original extension reaction and 0.75 units of Taq polymerase are added to each well.

Amplification generally was performed using the following conditions:

- Step 1 94°C for 60sec
- 10 Step 2 94°C for 20sec
- Step 3 55°C for 30sec
- Step 4 72°C for 90sec
- Step 5 repeat steps 2-4 for an additional 29 times
- Step 6 72°C for 180sec
- 15 Step 7 4°C for ever

Aliquots of these PCR reactions are run on agarose gels together with molecular weight markers. The size of the resulting PCR products will allow direct determination of how much additional sequence the selected clones contain compared to the original partial cDNA. The efficiency of the method has been further improved by using the resulting PCR-products directly for sequencing thus avoiding the necessity of preparing plasmids.

The appropriate clones are selected and grown for plasmid preparation and sequencing.

25 Plasmid preparations are made with standard kits familiar to those skilled in the art. Examples include the PROMEGA Magic MINIPREP and the AGTC alkaline lysis kit.

Sequencing is performed employing standard automated ABI sequencing equipment and protocols using either dye-primer or dye-terminator kits.

30 Sequence processing and assemblage of the sequencing data are performed using standard ABI software, including INHERIT™ analysis and the Power assembler.

INDUSTRIAL APPLICABILITYExample 1

For the initial method evaluation, a known gene was selected. A partial sequence of the human 90-kDa heat-shock protein gene (HUMHSP90, accession M16660) had been identified in a THP-1 library. This partial sequence (Incyte clone T-014201) initiated at base 1127 of the sequence with accession number M16660.

1.1 Primer design

Two primers were designed to perform the method described in the invention.

Primer 1 (XLR) 5' AGC TGT CCA TGA TGA ACA CAC G 3'
(1180-1159)

Primer 2 (XLS) 5' AAT AGG CAC CAC ACC AAC TGA G 3'
(2011-2032)

1.2 Template preparation

A THP-1 cDNA library constructed into the LambdaZAP vector (Stratagene) was converted into a plasmid library following the mass excision protocol. Plasmids of the excised libraries were prepared using the Quiagen Midi plasmid purification kit.

1.3 XL-PCR reaction set-up

The extension reactions were prepared following the instructions provided with the GeneAmp XL PCR Kit (Part No. N808-0182) from Perkin Elmer. A two layer system was set up as follows:

The lower reagent mix was prepared by pipetting the following components into a 0.2ml MicroAmp reaction tube.

Lower reagent mix preparation:

Water	13.6 μ l
3.3X buffer	12.0 μ l
dATP (10mM)	2.0 μ l
dCTP (10mM)	2.0 μ l

	dGTP	(10mM)	2.0 µl
	dTTP	(10mM)	2.0 µl
	Primer XLS	(50µM)	1.0 µl
	Primer XLR	(50µM)	1.0 µl
5	Mg(OAc)2	(25mM)	4.4 µl

Total lower reagent mix 40.0 µl

One AmpliWax™ gem was added to the tube. The wax was melted
10 by incubating the reaction tubes at 75°C for 5 minutes. Then the
tubes were cooled down to 4°C.

Upper reagent mix preparation:

	3.3X buffer	18.0 ml
15	rTth DNA Polymerase	2.0 ml

Total upper enzyme mix 20.0 µl

20 µl of the enzyme/buffer mix are added to each tube and
20 kept separated from the lower mix by the wax layer.

Addition of template:

The template DNA (excised library) was diluted to an
appropriate concentration in water and then added to the upper
mix. Mixing of the components is not necessary.

25

Template (6.25ng/ml)	40.0 µl
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Final volume 100.0 µl

30 1.4 XI-PCR amplification

For amplification the following protocol was employed:

- Step 1 94° for 60 sec (initial denaturation)
Step 2 94° for 15 sec
Step 3 65° for 1 min
Step 4 68° for 7 min
5 Step 5 Repeat step 2-4 for 15 additional times
Step 6 94° for 15 sec
Step 7 65° for 1 min
Step 8 68° for 7 min + 15 sec/cycle
Step 9 Repeat step 6-8 for 11 additional times
10 Step 10 72° for 8 min
Step 11 4° for 0.00 sec (to hold at 4°)

1.5 Purification of amplified products

30 µl of the amplified products were run on a 0.7% agarose
15 gel for 16 hours. Visible DNA bands were then cut out and purified
using the QIAquick gel purification kit.

1.6 Cloning of amplified products

Klenow enzyme (3 units/reaction) and dNTP's (0.2mM final
concentration) were added and the reactions were incubated at room
20 temperature for 30 min followed by incubation at 75° C for 15 min.
The products were then ethanol precipitated and redissolved in 13
µl of ligation buffer containing 1mM ATP. T4-DNA ligase (15 units)
and T4 Polynucleotide kinase (5 units) were added, and the
reaction was incubated at room temperature for 3 hours.

25 3µl of the ligation mixture were transformed into 40 ml of
competent E.coli cells. After heatshocking the cells at 42° C for
45 seconds, 80 µl of SOC medium were added, and the cells were
allowed to recover at 37° C for 1 hour. The whole transformation
mixture then was plated on LB-agar/2XCarb-containing petri dish
30 plates.

1.7 Screening of cloned products

The next day 10 colonies were randomly picked and grown

overnight in Falcon 2059 tubes (Becton Dickinson, Oxnard, CA) containing 3 ml of LB-broth with 2X Carb.

5 μ l of the cultures were diluted 1:10 with water and 5 ml of this dilution were transferred into MicroAmp™ PCR tubes (Perkin Elmer, Applied Biosystems, Foster City, CA).

15 μ l of a 1.33X concentrated PCR mix were added to each well.

The 1.33 x concentrated PCR mix contained the following components:

10	10X PCR-buffer	2.0 μ l
	2mM dNTPs	2.0 μ l
	M13 rev primer (0.01mM)	1.0 μ l
	Primer 2 (XLR, 0.01mM)	1.0 μ l
	Taq Polymerase	0.15 μ l
15	Water	8.85 μ l

Final Volume	15.0 μ l
--------------	--------------

The PCR cycling conditions were chosen as follows:

	Step 1	94° C for 60sec
20	Step 2	94° C for 20sec
	Step 3	55° C for 30sec
	Step 4	72° C for 90sec
	Step 5	repeat steps 2-4 for an additional 29 times
	Step 6	72° C for 180 sec
25	Step 7	4° C for ever

Aliquots of the amplified products were run on a 0.8% agarose gel in parallel with the 1 kb DNA ladder (Life Technologies, Gaithersburg, MD 20897). Appropriate plasmids containing different size inserts were selected for sequencing analysis.

30 1.8 Sequencing analysis of cloned products

The DNA of the selected clones was prepared using the

WizardTM Minipreps DNA Purification System (Promega Corporation, Madison, WI) following the instructions of the manufacturer. Sequencing reactions were performed using the PRISMTM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit (Part No 401628, Perkin Elmer, Applied Biosystems, Foster City, CA).

1.9 Analysis of sequenced products

Three clones were selected for sequencing (14201.3, 14201.5, 14201.13). The sequences obtained (SEQ ID NOS:3-5, respectively) were aligned using the DNASIS Multiple sequence alignment program. Clone 14201.3 initiated at base 24 of the published sequence (HUMHSP90), clone 14201.5 initiated at base 13 of the published sequence and clone 14201.13 initiated at base 538 of the published sequence, the original clone (14201) initiated at base 1127 of the published sequence.

Figure 7A-7H shows an alignment of the obtained sequences with the published human Hsp 90 nucleotide sequence. Clones 14201.3 and 14201.5 contain part of the 5' untranslated region and therefore the full coding region of the gene has been obtained.

Example 2

For further method evaluation, a second known gene was selected. A partial sequence from a liver library was found to be related to that of the human cathepsin B gene (accession L16510, HUMCATHB, SEQ ID NO:6). This partial sequence (Incyte clone 87058, SEQ ID NO:7) initiated at base 1066 of the sequence with accession number L16510.

2.1 Primer design

Two primers were designed to perform the method described in the invention:

Primer 1 (XLR) 5' AAG CCA TTG TCA CCC CAG TCA G 3'
(1103-1082)

Primer 2 (XLS) 5' GGT TCA CTG TGG AAT CGA ATC 3'
(1125-1145)

2.2 Template preparation

A liver cDNA library constructed into the LambdaZAP vector (Stratagene) was converted into a plasmid library following the mass excision protocol. Plasmids of the excised libraries were prepared using the Quiagen Midi plasmid purification kit.

5 2.3 XL-PCR reaction set-up

The extension reactions were prepared following the instructions provided with the GeneAmp XL PCR Kit (Part No. N808-0182) from Perkin Elmer. A two layer system was set up as described below. The lower reagent mix was prepared by pipetting
10 the following components into a 0.2ml MicroAmp reaction tube.

Lower reagent mix preparation:

	Water	13.6 μ l
	3.3 x buffer	12.0 μ l
	dATP (10mM)	2.0 μ l
15	dCTP (10mM)	2.0 μ l
	dGTP (10mM)	2.0 μ l
	dTTP (10mM)	2.0 μ l
	Primer XLS (50 μ M)	1.0 μ l
	Primer XLR (50 μ M)	1.0 μ l
20	Mg(OAc)2 (25 μ M)	4.4 μ l
<hr/>		
	Total lower reagent mix	40.0 μ l

One AmpliWax[®] gem was added to the tube. This was melted by
25 incubating the reaction tubes at 75°C for 5 minutes. Then the tubes were cooled down to 4°C.

Upper reagent mix preparation:

	3.3X buffer	18.0 μ l
30	rTth DNA Polymerase	2.0 μ l

Total upper enzyme mix 20.0 μ l

20 μ l of the enzyme/buffer mix were added to each tube and kept separated from the lower mix by the wax layer.

5 Addition of template:

The template DNA (excised library) was diluted to an appropriate concentration in water and then added to the upper mix. Mixing of the components is not necessary.

Template (6.25ng/ μ l) 40.0 μ l

10

Final volume 100.0 μ l

2.4 XL-PCR amplification

For amplification the following protocol was employed:

- Step 1 94° for 60 sec (initial denaturation)
- 15 Step 2 94° for 15 sec
- Step 3 65° for 1 min
- Step 4 68° for 7 min
- Step 5 Repeat step 2-4 for 15 additional times
- Step 6 94° for 15 sec
- 20 Step 7 65° for 1 min
- Step 8 68° for 7 min + 15 sec/cycle
- Step 9 Repeat step 6-8 for 11 additional times
- Step 10 72° for 8 min
- Step 11 4° for 0.00 sec (to hold at 4°)

25 2.5 Purification of amplified products

30 μ l of the amplified products were run on a 0.7% agarose gel for 16 hours. Visible DNA bands were then cut out and purified using the QIAQuick gel purification kit.

2.6 Cloning of amplified products

- 30 Klenow enzyme (3 units/reaction) and dNTP's (0.2mM final concentration) were added, and the reactions were incubated at room temperature for 30 min followed by incubation at 75°C for 15

min.

The products were then ethanol precipitated and redissolved in 13 μ l of ligation buffer containing 1mM ATP. T4-DNA ligase (15 units) and T4 Polynucleotide kinase (5 units) were added, and the
5 reaction was incubated at room temperature for 3 hours.

3 μ l of the ligation mixture were transformed into 40 μ l of competent E.coli cells. After heatshocking the cells at 42°C for 45 seconds, 80 μ l of SOC medium were added; and the cells were allowed to recover at 37°C for 1 hour. The whole transformation
10 mixture then was plated on LB-agar 2x Carb-containing petri dishes.

2.7 Screening of cloned products

The next day 10 colonies were randomly picked and grown overnight in Falcon 2059 tubes (Becton Dickinson, Oxnard, CA
15 93030) containing 3 ml of LB-broth with 2X Carb.

5 μ l of the cultures were diluted 1:10 with water and 5 μ l of this dilution were transferred into MicroAmpTM PCR tubes (Perkin Elmer, Applied Biosystems, Foster City, CA).

15 μ l of a 1.33 x concentrated PCR mix were added to each
20 tube.

The 1.33 x concentrated PCR mix contained the following components:

10 x PCR-buffer	2.0 μ l
2mM dNTPs	2.0 μ l
25 M13 rev primer (0.01mM)	1.0 μ l
Primer 2 (XLR, 0.01mM)	1.0 μ l
Taq Polymerase	0.15 μ l
water	8.85 μ l
<hr/>	
30 Final Volume	15.0 μ l

The PCR cycling conditions were as follows:

- Step 1 94°C for 60sec
Step 2 94°C for 20sec
Step 3 55°C for 30sec
Step 4 72°C for 90sec
5 Step 5 repeat steps 2-4 for an additional 29 times
Step 6 72°C for 180sec
Step 7 4°C for ever

Aliquots of the amplified products were run on a 0.8% agarose gel in parallel with the 1kb DNA ladder (Life Technologies,
10 Gaithersburg, MD 20897). Appropriate clones containing different size inserts were selected for sequencing analysis.

2.8 Sequencing analysis of cloned products

The DNA of the selected clones was prepared using the Wizard™ Minipreps DNA Purification System (Promega Corporation,
15 Madison, WI) following the instructions of the manufacturer. Sequencing reactions were performed using the PRISMTM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit (Part No 401628, Perkin Elmer, Applied Biosystems, Foster City, CA).

2.9 Analysis of sequenced products

20 Three clones were selected for sequencing (87058.6, 87058.8, 87058.16). The sequences obtained (SEQ ID NOS:8-10, respectively) were aligned using the DNASIS Multiple sequence alignment program and are shown in Figures 8A through 8F. Clone 87058.6 initiated at base 644 of the published sequence (HUMCATHB, SEQ ID NO:6),
25 clone 87058.8 initiated at base 353 of the published sequence and clone 87058.16 initiated at base 58 of the published sequence, the original clone (87058, SEQ ID NO:7) initiated at base 1058 of the published sequence.

Figures 8A through 8F show an alignment of the obtained
30 sequences with the published human Hsp 90 nucleotide sequence. Clone 87058.16 contains part of the 5'UT and therefore the full coding region of the gene.

Example 3

In Example 3, a full length cDNA (Seq ID NO 11) of a novel P2U purinergic receptor homolog was obtained by the inventive method and is the subject of U.S. Patent Application 08/459,046 filed June 2, 1995, which is hereby incorporated by reference.

5 Inherit™ and BLAST search and alignment tools were used to relate a partial sequence found in Incyte Clone 179696 from the placental cDNA library to the GenBank sequence of RNU09402, a G-protein coupled surface receptor from rat (Rice WR et al (1995) Am J Respir Cell Molec Biol 12:27-32).

10 The cDNA of Incyte 179696 was extended to full length using a modified XL-PCR (Perkin Elmer) procedure. Primers were designed based on known sequence; one primer was synthesized to initiate extension in the antisense direction (XLR) and the other to extend sequence in the sense direction (XLF). The primers allowed the
15 sequence to be extended "outward" from the known sequence, thus generating amplicons containing new, unknown nucleotide sequence comprising the gene of interest. The primers were designed using Oligo 4.0 (National Biosciences Inc, Plymouth MN) to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to
20 anneal to the target sequence at temperatures about 68°-72° C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations was avoided.

The cDNA library was used as a template, and XLR (bases 278-298) and XLF (bases 587-610) primers were used to extend and
25 amplify the 179696 sequence. By following the instructions for the XL-PCR kit and thoroughly mixing the enzyme, high fidelity amplification is obtained. Beginning with 25 pMol of each primer and the recommended concentrations of all other components of the kit, PCR was performed using the MJ PTC200 thermocycler (MJ
30 Research, Watertown MA) and the following parameters:

Step 1	94° C for 60 sec (initial denaturation)
Step 2	94° C for 15 sec
Step 3	65° C for 1 min

- Step 4 68° C for 7 min
Step 5 Repeat step 2-4 for 15 additional cycles
Step 6 94° C for 15 sec
Step 7 65° C for 1 min
5 Step 8 68° C for 7 min + 15 sec/cycle
Step 9 Repeat step 6-8 for 11 additional cycles
Step 10 72° C for 8 min
Step 11 4° C (and holding)

At the end of 28 cycles, 50 µl of the reaction mix was
10 removed; and the remaining reaction mix was run for an additional
10 cycles as outlined below:

- Step 1 94° C for 15 sec
Step 2 65° C for 1 min
Step 3 68° C for (10 min + 15 sec)/cycle
15 Step 4 Repeat step 1-3 for 9 additional cycles
Step 5 72° C for 10 min

A 5-10 µl aliquot of the reaction mixture was analyzed by
electrophoresis on a low concentration (about 0.6-0.8%) agarose
mini-gel to determine which reactions were successful in extending
20 the sequence. Although all extensions potentially contain a full
length gene, some of the largest products or bands were selected
and cut out of the gel. Further purification involved using a
commercial gel extraction method such as QIAQuick™ (QIAGEN Inc,
Chatsworth CA). After recovery of the DNA, Klenow enzyme was used
25 to trim single-stranded, nucleotide overhangs creating blunt ends
which facilitated religation and cloning.

After ethanol precipitation, the products were redissolved in
13 µl of ligation buffer. Then, 1µl T4-DNA ligase (15 units) and
1µl T4 polynucleotide kinase were added, and the mixture was
30 incubated at room temperature for 2-3 hours or overnight at 16° C.
Competent E. coli cells (in 40 µl of appropriate media) were
transformed with 3 µl of ligation mixture and cultured in 80 µl of
SOC medium (Sambrook J et al, supra). After incubation for one

hour at 37° C, the whole transformation mixture was plated on Luria Broth (LB)-agar (Sambrook J et al, supra) containing carbenicillin at 25 mg/L. The following day, 12 colonies were randomly picked from each plate and cultured in 150 µl of liquid LB/carbenicillin medium placed in an individual well of an appropriate, commercially-available, sterile 96-well microtiter plate. The following day, 5 µl of each overnight culture was transferred into a non-sterile 96-well plate and after dilution 1:10 with water, 5 µl of each sample was transferred into a PCR array.

For PCR amplification, 15 µl of concentrated PCR reaction mix (1.33X) containing 0.75 units of Taq polymerase, a vector primer and one or both of the gene specific primers used for the extension reaction were added to each well. Amplification was performed using the following conditions:

Step 1	94° C for 60 sec
Step 2	94° C for 20 sec
Step 3	55° C for 30 sec
Step 4	72° C for 90 sec
Step 5	Repeat steps 2-4 for an additional 29 cycles
Step 6	72° C for 180 sec
Step 7	4° C (and holding)

Aliquots of the PCR reactions were run on agarose gels together with molecular weight markers. The sizes of the PCR products were compared to the original partial cDNAs, and appropriate clones were selected, ligated into plasmid and sequenced.

Example 4

In this example, the inventive method was used to obtain a novel full length cDNA from the partial sequence found in Incyte clone 08118 which was found to be somewhat homologous to the GenBank sequence of C5a anaphylatoxin receptor, a G-protein coupled surface receptor from dog (Perret J et al (1995) Biochem

J 288:911-17). Based on the partial cDNA sequence, primers (XLR = GAAAGACAGCCACCACCACG and XLF = AGAAAGCAAGGCAGTCCATTCAGG) were designed. Essentially the same method outlined in Example 3 above was used to extend the partial sequence of 8118 to obtain the full length sequence (Seq ID NO:12) of a novel C5a-like receptor homolog which is the subject of a U.S. Patent Application 08/462,355 filed June 5, 1995, and whose disclosure is incorporated by reference.

While the present invention has been described with reference to specific enzymes and sequences, particularly PCR enzyme, and formulations containing such, those skilled in the art understand that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, enzyme, process, process step or steps and still carry out the objective, spirit and scope of the invention. All such modifications are intended to be within the scope of the claims appended hereto.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: INCYTE PHARMACEUTICALS, INC.
- (ii) TITLE OF INVENTION: IMPROVED METHOD FOR OBTAINING
FULL LENGTH CDNA SEQUENCES
- (iii) NUMBER OF SEQUENCES: 12
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: INCYTE PHARMACEUTICALS, INC.
 - (B) STREET: 3330 Hillview Avenue
 - (C) CITY: Palo Alto
 - (D) STATE: CA
 - (E) COUNTRY: USA
 - (F) ZIP: 94304
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: To Be Assigned
 - (B) FILING DATE: Filed Herewith
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION SERIAL NO: US 08/487,112
 - (B) FILING DATE: 7-JUN-1995
- (viii) PRIOR APPLICATION DATA:
 - (A) APPLICATION SERIAL NO: US 08/462,355
 - (B) FILING DATE: 5-JUN-1995
- (ix) PRIOR APPLICATION DATA:
 - (A) APPLICATION SERIAL NO: US 08/459,046
 - (B) FILING DATE: 2-JUN-1995
- (x) PRIOR APPLICATION DATA:
 - (A) APPLICATION SERIAL NO: US 08/566,334
 - (B) FILING DATE: 1-DEC-1995
- (xi) PRIOR APPLICATION DATA:
 - (A) APPLICATION SERIAL NO: US 60/006,809
 - (B) FILING DATE: 15-NOV-1995
- (xii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Luther, Barbara J.
 - (B) REGISTRATION NUMBER: 33954
 - (C) REFERENCE/DOCKET NUMBER: HP-001-1 PCT
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 415-855-0555

(B) TELEFAX: 415-852-0195

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2543 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:

- (A) LIBRARY: GenBank HUMHSP90
- (B) CLONE: Accession No. M16660

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CTCCGGCGCA GTGTTGGGAC TGTCTGGGTA TCGGAAAGCA AGCCTACGTT GCTCACTATT	60
ACGTATAATC CTTTTCTTTT CAAGATGCCT GAGGAAGTGC ACCATGGAGA GGAGGAGGTG	120
GAGACTTTTG CCTTTCAGGC AGAAATTGCC CAACTCATGT CCCTCATCAT CAATACCTTC	180
TATTCCAACA AGGAGATTTT CCTTCGGGAG TTGATCTCTA ATGCTTCTGA TGCCTTGGAC	240
AAGATTCGCT ATGAGAGCCT GACAGACCCT TCGAAGTTGG ACAGTGGTAA AGAGCTGAAA	300
ATTGACATCA TCCCCAACCC TCAGGAACGT ACCCTGACTT TGGTAGACAC AGGCATTGGC	360
ATGACCAAAG CTGATCTCAT AAATAATTTG GGAACCATTG CCAAGTCTGG TACTAAAGCA	420
TTCATGGAGG CTCTTCAGGC TGGTGCAGAC ATCTCCATGA TTGGGCAGTT TGGTGTGGC	480
TTTTATTCTG CCTACTTGGT GGCAGAGAAA GTGGTTGTGA TCAGAAAGCA CAACGATGAT	540
GAACAGTATG CTTGGGAGTC TTCTGCTGGA GGTTCCTTCA CTGTGCGTGC TGACCATGGT	600
GAGCCCATTG GCATGGGTAC CAAAGTGATC CTCCATCTTA AAGAAGATCA GACAGAGTAC	660
CTAGAAGAGA GGCGGGTCAA AGAAGTAGTG AAGAAGCATT CTCAGTTCAT AGGCTATCCC	720
ATCACCCCTT ATTTGGAGAA GGAACGAGAG AAGGAAATTA GTGATGATGA GGCAGAGGAA	780
GAGAAAGGTG AGAAAGAAGA GGAAGATAAA GATGATGAAG AAAAGCCCAA GATCGAAGAT	840
GTGGGTTCAG ATGAGGAGGA TGACAGCGGT AAGGATAAGA AGAAGAAAAC TAAGAAGATC	900
AAAGAGAAAT ACATTGATCA GGAAGAACTA AACAAGACCA AGCCTATTTG GACCAGAAAC	960
CCTGATGACA TCACCAAGA GGAGTATGGA GAATTCTACA AGAGCCTCAC TAATGACTGG	1020
GAAGACCACT TGGCAGTCAA GCACTTTTCT GTAGAAGGTC AGTTGGAATT CAGGGCATTG	1080
CTATTTATTC CTCGTCGGGC TCCCTTTGAC CTTTTTGAGA ACAAGAAGAA AAAGAACAAC	1140
ATCAAACCTCT ATGTCCGCCG TGTGTTTCATC ATGGACAGCT GTGATGAGTT GATACCAGAG	1200

TATCTCAATT TTATCCGTGG TGTGGTTGAC TCTGAGGATC TGCCCCTGAA CATCTCCCGA	1260
GAAATGCTCC AGCAGAGCAA AATCTTGAAA GTCATTGCGA AAAACATTGT TAAGAAGTGC	1320
CTTGAGCTCT TCTCTGAGCT GGCAGAAGAC AAGGAGAATT ACAAGAAATT CTATGAGGCA	1380
TTCTCTAAAA ATCTCAAGCT TGGAATCCAC GAAGACTCCA CTAACCGCCG CCGCCTGTCT	1440
GAGCTGCTGC GCTATCATAC CTCCCAGTCT GGAGATGAGA TGACATCTCT GTCAGAGTAT	1500
GTTCCTCGCA TGAAGGAGAC ACAGAAGTCC ATCTATTACA TCACTGGTGA GAGCAAAGAG	1560
CAGGTGGCCA ACTCAGCTTT TGTGGAGCGA GTGCGGAAAC GGGGCTTCGA GGTGGTATAT	1620
ATGACCGAGC CCATTGACGA GTACTGTGTG CAGCAGCTCA AGGAATTTGA TGGGAAGAGC	1680
CTGGTCTCAG TTACCAAGGA GGGTCTGGAG CTGCCTGAGG ATGAGGAGGA GAAGAAGAAG	1740
ATGGAAGAGA GCAAGGCAAA GTTTGAGAAC CTCTGCAAGC TCATGAAAGA AATCTTAGAT	1800
AAGAAGGTTG AGAAGGTGAC AATCTCCAAT AGACTTGTGT CTTCACCTTG CTGCATTGTG	1860
ACCAGCACCT ACGGCTGGAC AGCCAATATG GAGCGGATCA TGAAAGCCCA GGCACCTCGG	1920
GACAACTCCA CCATGGGCTA TATGATGGCC AAAAAGCACC TGGAGATCAA CCCTGACCAC	1980
CCCATTGTGG AGACGCTGCG GCAGAAGGCT GAGGCCGACA AGAATGATAA GGCAGTTAAG	2040
GACCTGGTGG TGCTGCTGTT TGAAACCGCC CTGCTATCTT CTGGCTTTTC CCTTGAGGAT	2100
CCCCAGACCC ACTCCAACCG CATCTATCGC ATGATCAAGC TAGGTCTAGG TATTGATGAA	2160
GATGAAGTGG CAGCAGAGGA ACCCAATGCT GCAGTTCCTG ATGAGATCCC CCCTCTCGAG	2220
GGCGATGAGG ATGCGTCTCG CATGGAAGAA GTCGATTAGG TTAGGAGTTC ATAGTTGGAA	2280
AACTTGTGCC CTTGTATAGT GTCCCCATGG GCTCCCACTG CAGCCTCGAG TGCCCCGTGC	2340
CCACCTGGCT CCCCCTGCTG GTGTCTAGTG TTTTTCCTCC TCTCCTGTCC TTGTGTTGAA	2400
GGCAGTAAAC TAAGGGTGTC AAGCCCCATT CCCTCTCTAC TCTTGACAGC AGGATTGGAT	2460
GTTGTGTATT GTGGTTTATT TTATTTTCTT CATTTTGTTT TGAAATTAAA GTATGCAAAA	2520
TAAAGAATAT GCCGTTTTTA TAC	2543

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 261 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:

- (A) LIBRARY: THP-1
- (B) CLONE: 14201

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

AAGAAAAAGA ACAACATCAA ACTCTATGTC CGCCGTGTGT TCATCATGGC AGCTGTGATG      60
AGTTGATAACC AGAGTATCTC AATTTTATCC GTGGTGTGGT TGAATTGAGG TCTGCCCCTG     120
AACATCTCCC GGAAATGCTC CAGCAGAGCA AAATCTTGAA AGGCATTTCG AAAACATTG      180
TTAAGAGTGC CTTAGCTCTT CTCTAGCTGG CAGAAGCAAG GGGATTTCAG GAAATTCTTT     240
TGGGGGGGATT TCTTAAAAAT T                                              261

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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 478 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:

- (A) LIBRARY: THP-1
- (B) CLONE: 14201.3

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```

GCTGGGTATC GGAAAGCAAG CCTACGTTGC TCACTATTAC GTATAATCCT TTTCTTCAAG      60
ATGCCTGAGG AAGTGCACCA TGGAGAGGAG GAGGTGGAGA CTTTTCCTT TCAGGCAGAA     120
ATTGCCCAAC TCATGTCCCT CATCATCAAT ACCTCCTATT CCAACAAGGA GATTTCCTCG     180
GGAGTTGATC TCTAATGCTT CTGATGCCTC GGACAAGATT CGCTATGAAG CCTGACAGAC     240
CCTTCGAAGT GGTGAGCGGC AAGAGCTGAA AATTGACATC ATCCCAACC CTCAGGAACG      300
TCCCTGTACT TTGGGTAGAC ACAGGCATTG GCATAAACAA AGCTGACCTC ATATTATTCG     360
GGGAACCATT GCCAAGTCTT GTCTAAAAGC ATTCATGGAG GCTCTCAGGT TGGCGCAGAC     420
ATCTCCAGAT TGGCAGGTGG GTGTTGGCTT TATTCTGCCC ACTTGGTGGC AGAGAAAT      478

```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 508 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: THP-1

(B) CLONE: 14201.5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GTTGGGACTG TCTGGGTATC GGAAAGCAAG CCTACGTTGC TCACTATTAC GTATAATCCT	60
TTTCTTTTCA AGATGCCTGA GGAAGTGCAC CATGGAGAGG AGGAGGTGGA GACTTTTGCC	120
TTTCAGGCAG AAATTGCCCA ACTCATGTCC CTCATCATCA ATACCTCCTA TTCCAACAAG	180
GAGATTTTCC TTCGGGAGTT GATCTCTAAT GCTTCTGATG CCTTGGACAA GATTCGCTAT	240
GAGAGCCTGA CAGACCCTTC GAAGTTGGAC AGTGGTAAAG AGCTGAAAAT TGACATCATC	300
CCCAACCCTC AGGAACGTAC CCTGACTTTG GGTAGACACA GGCATCGGCA TGACCAAAG	360
CTGATCTCAT AATAATTGGG AACCATTGCA AGTCTGGTAC TAAAGCATTG ATGGAGGCTC	420
TTCAGGCTGG TGCAGACATC TCCATGATTG GGCAGCTTGG GTGTTGCTTT ATTCTGCCTC	480
CTTGGTGGCA GAGAAAGTGT TGTGATCA	508

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 547 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: THP-1

(B) CLONE: 14201.13

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TTGAGAGTAT GTCGAGTTAC TGTGGAGGTT CCTTCACTGC GTGCTGACAT GGTGAGCCCA	60
TGGGAGCGGT ACCAAGTGAT CCTCCATCTC AAAGAAGATC AGACAGAGTA CCTAGAGAGA	120
GGCGGATCAA AGAGTAGTGA TGAGCATCCT CAGATCATAG GCTATCCCAT CACCCTTTTT	180
TGGAGAAGGA CGAGAGAAGG AATTAGGATG ATGAGGCAGA GGAAGAGAAT GGTGAGAATG	240
AAGAGGAGTA ACGATGATGA AGAAACCCCA AGATCGATGA TGTGGTTCAG ATGAGGGGAT	300
GACAGCGGTA GATAAGAAGA AGAACTAGA ATCATCGGAT CATGACAGGA AGAACTAACA	360
GATCATCTTT CGGCCAGAAT CCCTGATGTC ATCACCAG AGGGTATGGA GATTTCTACA	420
TGCAGCTCAC TTTACTGGGC AAGACACTTG GCAGCAACAC TTTTCTGTAG AAGGCCATTG	480

CATCACGCAT TGCTATTCTT CCCTCGCCGT CTCCTTTGAC CTGGTCTGGC ATCATGGTGT 540
CTTGATC 547

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1996 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:

- (A) LIBRARY: GenBank HUMCATHB
- (B) CLONE: Accession No. L16510

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TCCGGCAACG CCAACCGCTC CGCTGCGCGC AGGCTGGGCT GCAGGCTCTC GGCTGCAGCG 60
CTGGGCTGGT GTGCAGTGGT GCGACCACGG CTCACGGCAG CCTCAGCCAC CCAGATGTAA 120
GCGATCTGGT TCCCACCTCA GCCTCCCAG TAGTGGATCT AGGATCCGGC TTCCAACATG 180
TGGCAGCTCT GGGCCTCCCT CTGCTGCCTG CTGGTGTGG CCAATGCCCG GAGCAGGCCC 240
TCTTTCCATC CCCTGTCGGA TGAGCTGGTC AACTATGTCA ACAAACGGAA TACCACGTGG 300
CAGGCCGGGC ACAACTTCTA CAACGTGGAC ATGAGCTACT TGAAGAGGCT ATGTGGTACC 360
TTCCTGGGTG GGCCCAAGCC ACCCCAGAGA GTTATGTTTA CCGAGGACCT GAAGCTGCCT 420
GCAAGCTTCG ATGCACGGGA ACAATGGCCA CAGTGTCCCA CCATCAAAGA GATCAGAGAC 480
CAGGGCTCCT GTGGCTCCTG CTGGGCCTTC GGGGCTGTGG AAGCCATCTC TGACCGGATC 540
TGCATCCACA CCAATGCGCA CGTCAGCGTG GAGGTGTCGG CGGAGGACCT GCTCACATGC 600
TGTGGCAGCA TGTGTGGGA CGGCTGTAAT GGTGGCTATC CTGCTGAAGC TTGGAAC TTC 660
TGGACAAGAA AAGGCCTGGT TTCTGGTGGC CTCTATGAAT CCCATGTAGG GTGCAGACCG 720
TACTCCATCC CTCCTGTGA GCACCACGTC AACGGCTCCC GGCCCCCATG CACGGGGGAG 780
GGAGATACCC CCAAGTGTAG CAAGATCTGT GAGCCTGGCT ACAGCCCGAC CTACAAACAG 840
GACAAGCACT ACGGATACAA TTCCTACAGC GTCTCCAATA GCGAGAAGGA CATCATGGCC 900
GAGATCTACA AAAACGGCCC CGTGGAGGGA GCTTTCTCTG TGTATTCGGA CTTCTGCTC 960
TACAAGTCAG GAGTGACCA ACACGTCACC GGAGAGATGA TGGGTGGCCA TGCCATCCGC 1020
ATCCTGGGCT GGGGAGTGGA GAATGGCACA CCCTACTGGC TGGTTGCCAA CTCCTGGAAC 1080
ACTGACTGGG GTGACAATGG CTTCTTTAAA ATACTCAGAG GACAGGATCA CTGTGGAATC 1140

GAATCAGAAG TGGTGGCTGG AATTCCACGC ACCGATCAGT ACTGGGAAAA GATCTAATCT	1200
GCCGTGGGCC TGTCGTGCCA GTCCTGGGGG CGAGATCGGG GTAGAAATGC ATTTTATTCT	1260
TTAAGTTCAC GTAAGATACA AGTTTCAGGC AGGGTCTGAA GGACTGGATT GGCCAAACAT	1320
CAGACCTGTC TTCCAAGGAG ACCAAGTCCT GGCTACATCC CAGCCTGTGG TTACAGTGCA	1380
GACAGGCCAT GTGAGCCACC GCTGCCAGCA CAGAGCGTCC TTCCCCCTGT AGACTAGTGC	1440
CGTGGGAGTA CCTGCTGCCC AGCTGCTGTG GCCCCCTCCG TGATCCATCC ATCTCCAGGG	1500
AGCAAGACAG AGACGCAGGA TGGAAAGCGG AGTTCCTAAC AGGATGAAAG TTCCCCCATC	1560
AGTTCCCCCA GTACCTCCAA GCAAGTAGCT TTCCACATTT GTCACAGAAA TCAGAGGAGA	1620
GATGGTGTG GGAGCCCTTT GGAGAACGCC AGTCTCCAGG TCCCCCTGCA TCTATCGAGT	1680
TTGCAATGTC ACAACCTCTC TGATCTGTG CTCAGCATGA TTCTTTAATA GAAGTTTTAT	1740
TTTTCGTGCA CTCTGCTAAT CATGTGGGTG AGCCAGTGGA ACAGCGGGAG CCTGTGCTGG	1800
TTTGCAGATT GCCTCCTAAT GACGCGGCTC AAAAGGAAAC CAAGTGGTCA GGAGTTGTTT	1860
CTGACCCACT GATCTCTACT ACCACAAGGA AAATAGTTTA GGAGAAACCA GCTTTTACTG	1920
TTTTTGAAAA ATTACAGCTT CACCCTGTCA AGTTAACAAG GAATGCCTGT GCCAATAAAA	1980
GGTTTCTCCA ACTTGA	1996

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 294 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vii) IMMEDIATE SOURCE:
- (A) LIBRARY: LIVER
 - (B) CLONE: 87058

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CGGCACGAGC CAACTCCTGG AACACTGACT GGGGTGACAA TGGCTTCTTT AAAATACTCA	60
GAGGACAGGT TCACTGTGGA ATCGAATCAG AAGTGGTGGC TGGAAATCCA CGCACC GTTC	120
AGTACTGGGA AAAGTCTAAT CTGCCGTGGG CCTTCGTGCC AGTCCTGGGG GCGAGATGGG	180
GGTAGAAATG CATTTTATTC TTTAAGTTCA CGTAAGATAC AAGTTTCAGA CAGGGGTCTA	240
AGGCCTGGTT GCCAAAATCA GACCTGTTTT TCAAGGGGCC CAAGTCCTGG GTTC	294

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 552 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vii) IMMEDIATE SOURCE:
 (A) LIBRARY: Liver
 (B) CLONE: 87058.6

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```

GTGAAGCTTG GAACTTCTGG ACAAGAAAAG GCCTGGTTTC TGGTGGCCTC TATGAATCCC      60
ATGTAGGGTG CAGACCGTAC TCCATCCCTC CCTGTGAGCA CCACGTCAAC GGCTCCCGGC      120
CCCCATGCAC GGGGGAGGGA GATACCCCA AGTGTAGCAA GATCTGTGAG CCTGGCTACA      180
GCCCGACCTA CAAACAGGAC AAGCACTACG GATACAATTC CTACAGCGTC TCCAATAGCG      240
AGAAGGACAT CATGGCCGAG ATCTACAAA ACGCCCCCGT GGAGGGAGCT TTCTCTGTGT      300
ATTCGGA CTTCTGCTCTAC AAGTCAGGAG TGTACCAACA CGTCACCGGA GAGATGATGG      360
GTGGCCATGC CATCCGCATC CTGGGCTGGG GAGTGGAGAA TGGCACAACC TACTGGCTGG      420
TTGGCAACTC CTGGAACACT GACTGGGGTG ACAATGGGTT CACTGTGGAA TCGAATCAGA      480
AGTGGTGGTG GAATTCCACG CACGATCAAG TGCTGGGAAA AGATCTTAAT CTGCCGGGGC      540
TGTCGGCCAG TC                                                              552

```

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 559 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vii) IMMEDIATE SOURCE:
 (A) LIBRARY: Liver
 (B) CLONE: 87058.8

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```

GAGGTACCTT CCTGGGTGGG CCCAAGCCAC CCCAGAGAGT TATGTTTACC GAGGACCTGA      60
AGCTGCCTGC AAGCTTCGAT GCACGGGAAC AATGGCCACA GTGTCCCACC ATCAAAGAGA      120
TCAGAGACCA GGGTCCTGTG GCTCCTGCTG GGCCTTCGGG GCTGTGGAAG CCATCTCTGA      180

```

CCGGATCTGA TCCACACCAA TGC GCACGTC AGCGTGGAGG TGTCGGCGGA GGACTGCTCA	240
CATGCTGTGG CAGATGTGTG GGGACGGCTG TAATGGTGGC TATCCTGCTG AAGCTTGGAC	300
TTCTGGACAA GAAAAGGCCC TGGTTTCTGG TGGCCTCTAT GATCCCATGT AGGGTGTAGA	360
CCGTACTCCA TCCCTCCCTG TGAAGCACCA CGTCAACGGT TCCCGGGCCC CATGCACGGG	420
GAGGGAGATA CCCCCAAGTG TAACAAGATC TGTGAGCCTG GGTACAGTCC CGACCACAAA	480
CAGGAAAAGC ACTACGGATA CAATTCCTCA GGTCTCCAAT AGTGAGAAGG GACATCATGC	540
CGAGATCTAC AATAACGGC	559

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 622 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:

- (A) LIBRARY: Liver
- (B) CLONE: 87058.16

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CGGTTGAGAT TCGGACAGTC CGAAAACGTC CGGCAAGTCA CCCGCTCCGC TGGCGCAGGC	60
TGGGTGCAGG CTCTCGGTGC AGGCTGGGTG GATCTAGGAT CCGGCTTCCA ACATGTGGCA	120
GTTCTGGGCC TCCCTCTGTG CCTGCTGGTG TTGACAATG CCCGGAGGAG GCCTCTTTCC	180
ATCCCCTGTC GGATGAGCTG GTCATATGT CAACAAACGG AATACCACGT GGAGGCCGGG	240
AACAATTCT ACAACGTGGA CATGAGCTAC TTGAGAGGTA TGTGGTACCT TCCTGGGTGG	300
GCCCAAGCCA CCCAGAGAG TTTGTTTACC GAGGACCTGA GCTGCCTGCA AGCTTCGAAG	360
GACGGGAACA ATGGCCACAG TGTCCACCA TCAAAGAGAT CAGAGACAGG GCTCCTGTGG	420
TCCTGCTGGG CCTCCGGGGC TGTGGAAGCA TCTCTGACCG GATCTGCATC CACACCAATG	480
GCACGTCAGC GTGGTGGTGT CGGGGAGGAC CTGATCACCT TTGTGGTAGC ATGTGTGGGG	540
GACGGCTGTA ATGGTGGTTA TCCTGTGAAG CTGGGCCTTC TAGAAAGAAA AGGCTGTTTT	600
GGTGGCCTTA TGA TCCCAT GT	622

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 984 base pairs

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:

- (A) LIBRARY: Placenta
- (B) CLONE: 179696

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ATGGAATGGG ACAATGGCAC AGACCAGGCT CTGGGCTTGC CACCCACCAC CTGTGTCTAC	60
CGCGAGAACT TCAAGCAACT GCTGCTCCCA CCTGTGTATT CGGCGGTGCT GGCGCCTGCC	120
CTCCCGCTGA ACATCTGTGT CATTACCCAG ATCTGCACGT CCCGCCGGGC CCTGACCCGC	180
ACGGCCGTGT ACACCCTAAA CCTTGCTCTG CCTGACCTGC TATATGCCTG CTCCCTGCCC	240
CTGCTCATCT ACAACTATGC CCAAGGTGAT CACTGGCCCT TTGGCGACTT CGCCTGCCGC	300
CTGGTCCGCT TCCTCTTCTA TGCCAACCTG CACGGGAGGA TCCTCTTCCT CACCTGCATC	360
AGCTTCCAGC GCTACCTGGG CATCTGCCAC CCGCTGGCCC CCTGGCACAA ACGTGGGGGC	420
CGCCGGGCTG CCTGGCTAGT GTGTGTAGCC GTGTGGCTGG CCGTGACAAC CCAGTGCCTG	480
CCCACAGCCA TCTTCGCTGC CACAGGCATC CAGCGTAACC GCACTGTCTG TTATGACCTC	540
AGCCCGCCTG CCCTGGCCAC CCACTATATG CCCTATGGGA TGGCTCTCAC TGTCATCGGC	600
TTCTGTCTGC CCTTTGCTGC CCTGCTGGCC TGCTACTGTC TCCTGGCCTG CCGCCTGTGC	660
CGCCAGGATG GCCCGGCAGA GCCTGTGGCC CAGGAGCGGC GTGGCAAGGC GGCCCGCATG	720
GCCGTGGTGG TGGCTGCTGT CTTTGGCATC AGCTTCCTGC CTTTTACAT CACCAAGACA	780
GCCTACCTGG CAGTGCCTC GACGCCGGGC GTCCCCTGCA CTGTATTGGA GGCCTTTGCA	840
GCGGCCTACA AAGGCACGCG GCCGTTTGCC AGTGCCAACA GCGTGCTGGA CCCCATCCTC	900
TTCTACTTCA CCCAGAAGAA GTCCGCCGG CGACCACATG AGCTCCTACA GAAACTCACA	960
GACAAATGGC AGAGGCAGGG TCGC	984

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1446 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: Mast Cell
(B) CLONE: 8118

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

ATGGCGTCTT TCTCTGCTGA GACCAATTCA ACTGACCTAC TCTCACAGCC ATGGAATGAG	60
CCCCAGTAA TTCTCTCCAT GGTCAATTCTC AGCCTTACTT TTTTACTGGG ATTGCCAGGC	120
AATGGGCTGG TGCTGTGGGT GGCTGGCCTG AAGATGCAGC GGACAGTGAA CACAATTTGG	180
TTCTCCACC TCACCTTGGC GGACCTCCTC TGCTGCCTCT CCTTGGCCTT CTCGCTGGCT	240
CACTTGGCTC TCCAGGGACA GTGGCCCTAC GGCAGGTTCC TATGCAAGCT CATCCCCTCC	300
ATCATTGTCC TCAACATGTT TGGCAGTGTC TTCCTGCTTA CTGCCATTAG CCTGGATCGC	360
TGTCTTGTGG TATTCAAGCC AATCTGGTGT CAGAATCATC GCAATGTAGG GATGGCCTGC	420
TCTATCTGTG GATGTATCTG GGTGGTGGCT TTTGTGTTGT GCATTCTGT GTTCGTGTAC	480
CGGGAAATCT TCACTACAGA CAACCATAAT AGATGTGGCT ACAAATTTGG TCTCTCCAGC	540
TCATTAGATT ATCCAGACTT TTATGGGGAT CCACTAGAAA ACAGGTCTCT TGAAAACATT	600
GTTCAGCCGC CTGGAGAAAT GAATGATAGG TTAGATCCTT CCTCTTTCCA AACAAATGAT	660
CATCCTTGA CAGTCCCCAC TGTCTTCCAA CCTCAAACAT TTCAAAGACC TTCTGCAGAT	720
TCACTCCCTA GGGGTCTGC TAGGTAAACA AGTCAAAATC TGTATTCTAA TGTATTTAAA	780
CCTGCTGATG TGGTCTCACC TAAAATCCCC AGTGGGTTTC CTATTGAAGA TCACGAAACC	840
AGCCCACTGG ATAACCTCTGA TGCTTTTCTC TCTACTCATT TAAAGCTGTT CCCTAGCGCT	900
TCTAGCAATT CCTTCTACGA GTCTGAGCTA CCACAAGGTT TCCAGGATTA TTACAATTTA	960
GGCCAATTCA CAGATGACGA TCAAGTGCCA ACACCCCTCG TGGCAATAAC GATCACTAGG	1020
CTAGTGGTGG GTTTCCTGCT GCCCTCTGTT ATCATGATAG CCTGTTACAG CTTTATTGTC	1080
TTCCGAATGC AAAGGGGCCG CTTCCGCAAG TCTCAGAGCA AAACCTTTTC AGTGGCCGTG	1140
GTGGTGGTGG CTGTCTTTCT TGTCTGCTGG ACTCCATACC ACATTTGGGG AGTCCTGTCA	1200
TTGCTTACTG ACCCAGAAAC TCCCTTGGGG AAAACTCTGA TGTCTGGGA TCATGTATGC	1260
ATTGCTCTAG CATCTGCCAA TAGTTGCTTT AATCCCTTCC TTTATGCCCT CTTGGGGAAA	1320
GATTTTAGGA AGAAAGCAAG GCAGTCCATT CAGGGAATTC TGGAGGCAGC CTTCAGTGAG	1380
GAGCTCACAC GTTCCACCCA CTGTCCCTCA AACAATGTCA TTTCAGAAAG AAATAGTACA	1440
ACTGTG	1446

CLAIMS

1. A method of extending the sequence of a partial complementary DNA (cDNA) using polymerase chain reaction (PCR), comprising the steps of:

5 a) combining a first and second PCR primer with nucleic acid from a cDNA library expected to contain said partial cDNA, or a genomic library, under conditions suitable for synthesis of nucleic acid PCR products from the first and second primers, wherein said first and second primers are capable of annealing to
10 opposite strands of the partial cDNA or genomic DNA and initiating nucleic acid synthesis in an outward manner and wherein the first primer is capable of being extended by DNA polymerase in an antisense direction and the second primer is capable of being extended in a sense direction.

15 b) purifying the PCR products, and

 c) identifying extended nucleotide sequences derived from said partial cDNA or said genomic DNA.

2. The method of Claim 1 wherein identifying extended sequences comprises nucleic acid sequencing.

20 3. The method of Claim 2 further comprising extending the nucleotide sequences of step 6c by repeating steps 6a through 6c on the nucleotide sequences identified in step 6c.

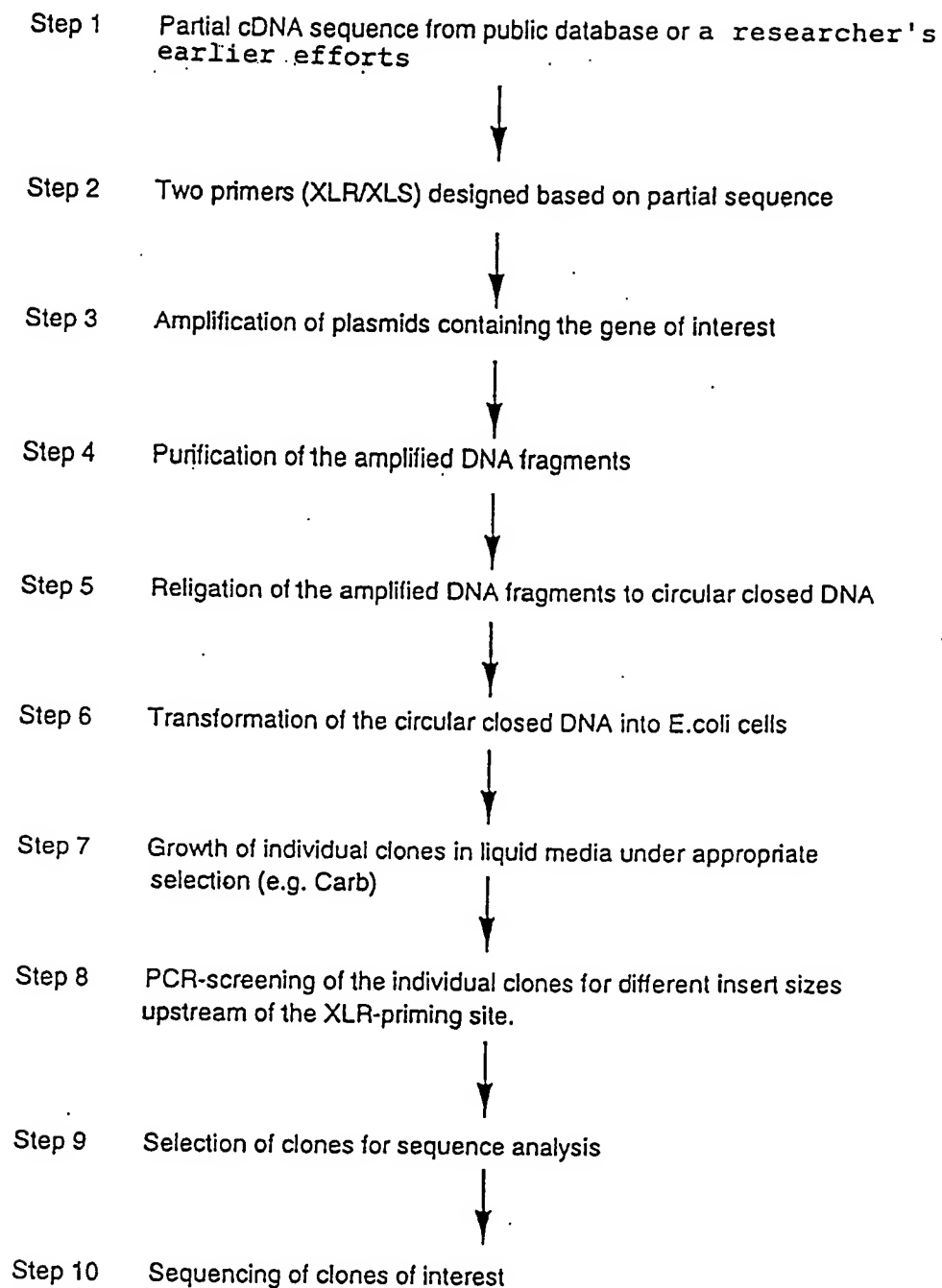
 4. A method of extending the nucleotide sequence of a partial complementary DNA (cDNA) using polymerase chain reaction
25 (PCR), comprising the steps of:

 a) combining a first and second PCR primer with nucleic acid from a cDNA library expected to contain said partial cDNA, or a genomic library, under conditions suitable for synthesis of nucleic acid PCR products from the first and second primers,
30 wherein said first and second primers are capable of annealing to opposite strands of the partial cDNA or genomic DNA and initiating nucleic acid synthesis in an outward manner and wherein the first primer is capable of being extended by DNA polymerase in an

antisense direction and the second primer is capable of being extended in a sense direction.

- b) purifying the PCR products,
 - c) ligating the purified PCR products under conditions
 - 5 suitable for the formation of circular closed nucleic acid,
 - d) transforming a host cell with the circular closed nucleic acid and culturing the transformed host cell under conditions suitable for growth,
 - e) recovering said circular closed nucleic acid from the
 - 10 cultured, transformed host cell,
 - f) identifying extended nucleotide sequences derived from said partial cDNA or said genomic DNA.
5. The method of Claim 4 wherein identifying extended sequences comprises nucleic acid sequencing.
- 15 6. The method of Claim 4 wherein culturing the transformed host cell under conditions suitable for growth comprises culturing in the presence of selective antibiotic conditions.
7. The method of Claim 4 wherein said host cell is E.coli.
8. The method of Claim 4 wherein after step 4b and prior to step
- 20 4c, the purified PCR products are treated under conditions suitable for converting nucleic acid overhangs to blunt ends.

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**FIGURE 1**

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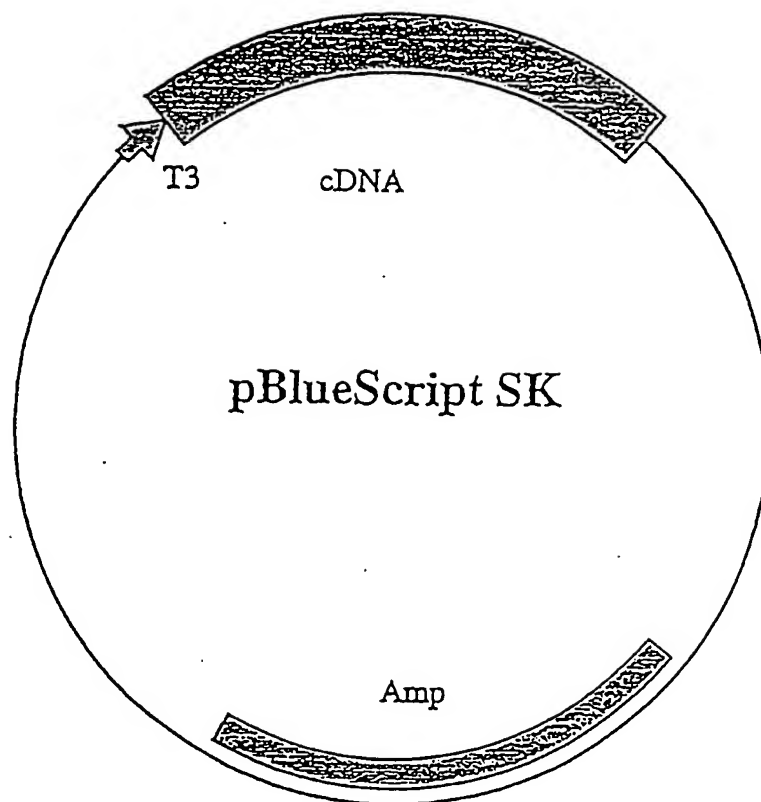
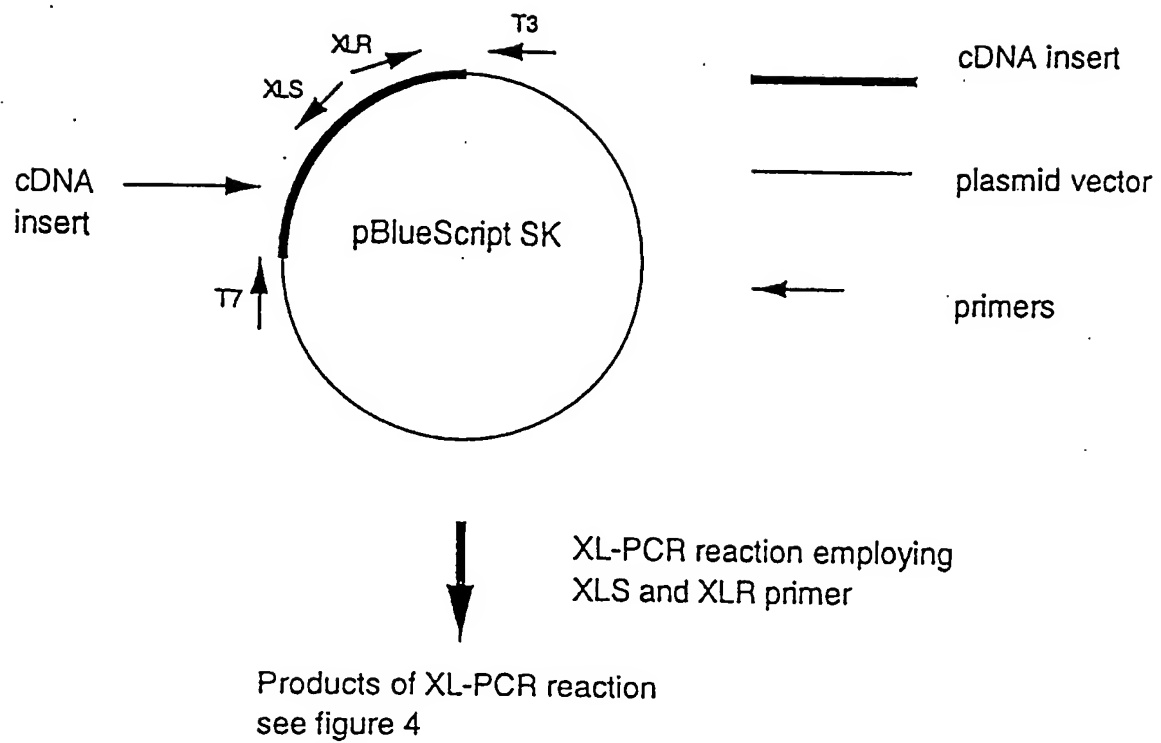
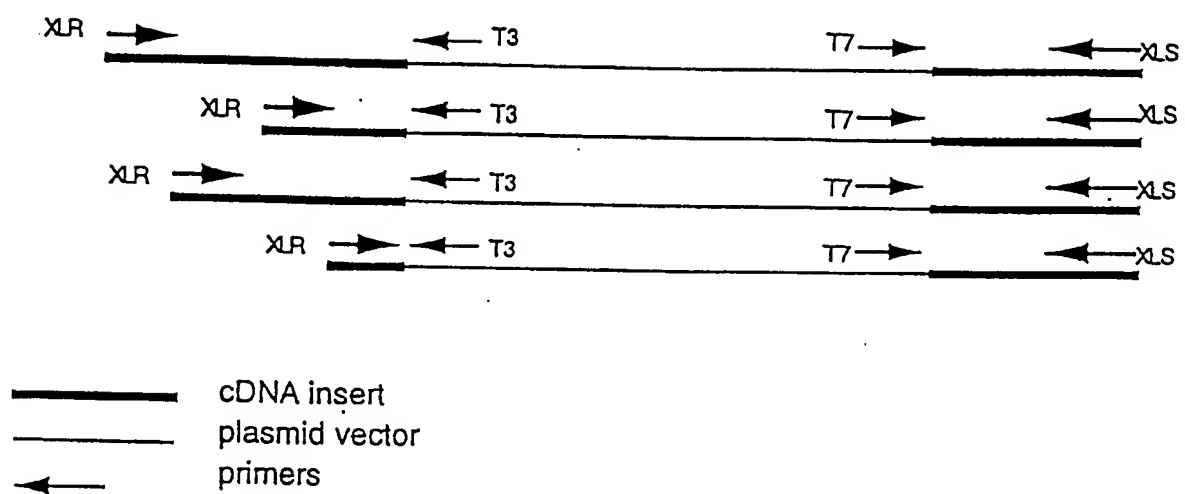


FIGURE 2

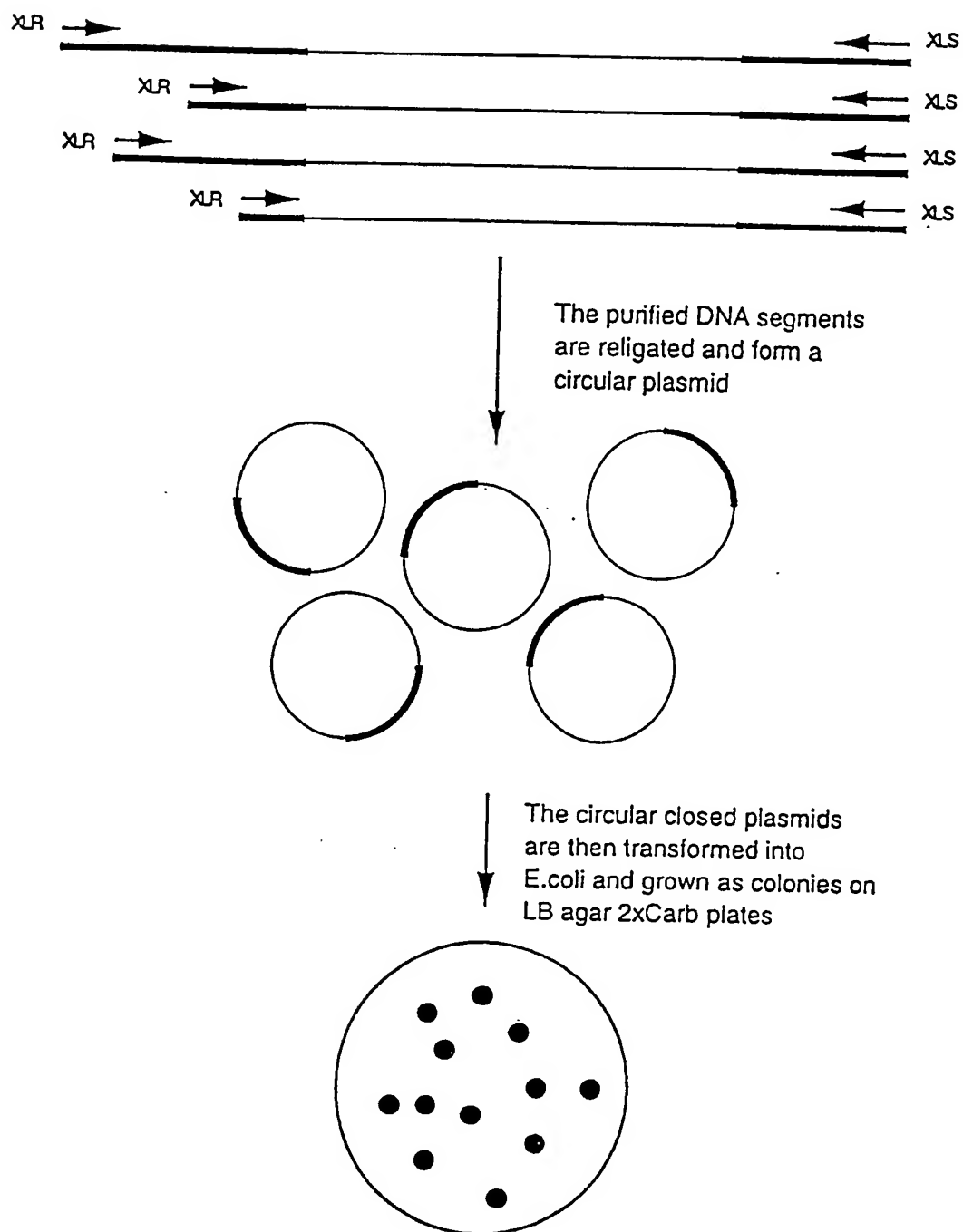
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**FIGURE 3**

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**FIGURE 4**

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**FIGURE 5**

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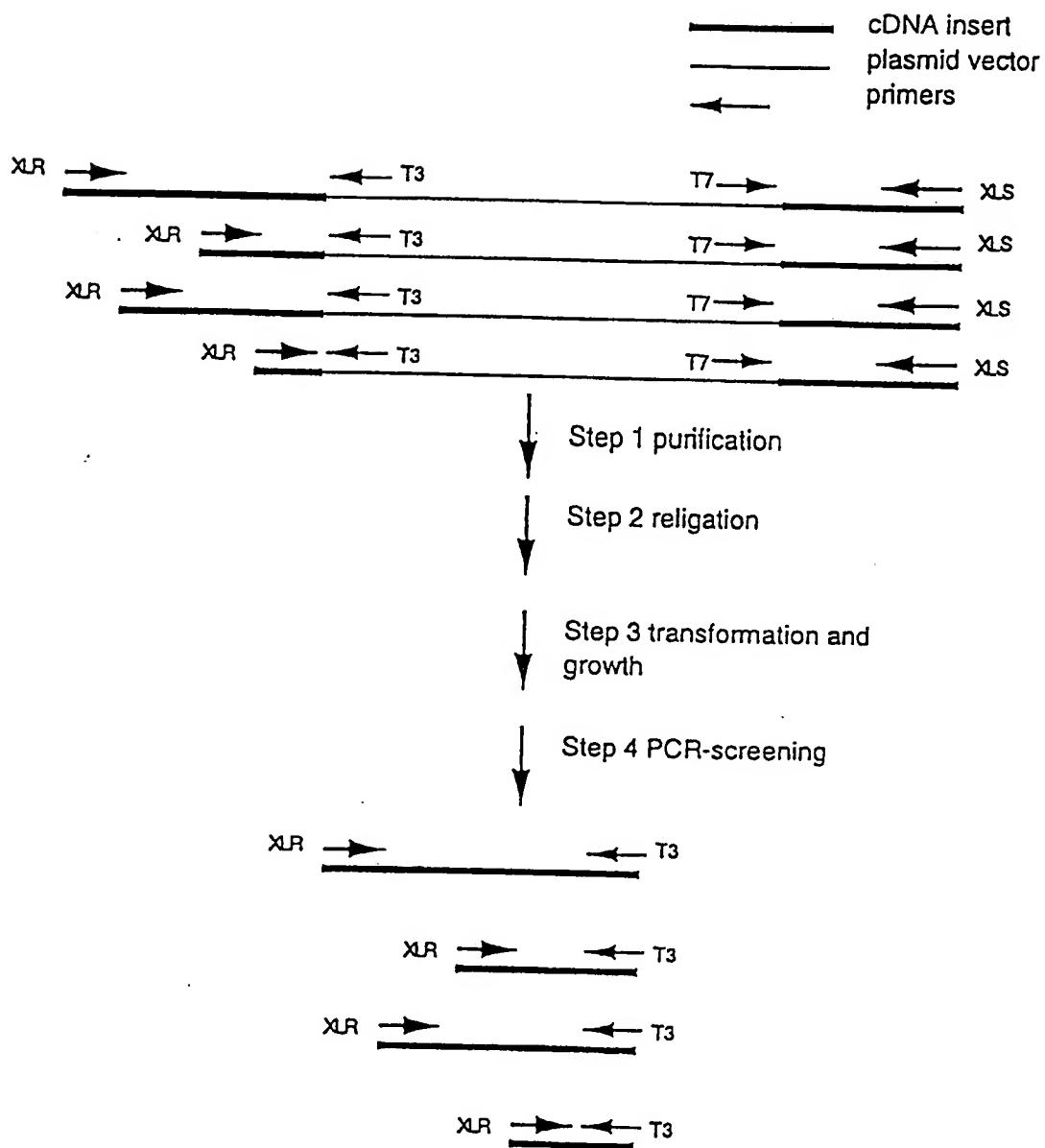


FIGURE 6

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		10	20	30	40	50	
Hsp 90	1	CTCCGGCGCA	GTGTTGGGAC	TGTCTGGGTA	TCGGAAAGCA	AGCCTACGTT	50
14201	1	-----	-----	-----	-----	-----	50
14201.3	1	-----	-----	--GCTGGGTA	TCGGAAAGCA	AGCCTACGTT	50
14201.5	1	-----	--GTTGGGAC	TGTCTGGGTA	TCGGAAAGCA	AGCCTACGTT	50
14201.13	1	-----	-----	-----	-----	-----	50
		60	70	80	90	100	
Hsp 90	51	GCTCACTATT	ACGTATAATC	CTTTTCTTTT	CAAGATGCCT	GAGGAAGTGC	100
14201	51	-----	-----	-----	-----	-----	100
14201.3	51	GCTCACTATT	ACGTATAATC	CTTTTCTNTN	CAAGATGCCT	GAGGAAGTGC	100
14201.5	51	GCTCACTATT	ACGTATAATC	CTTTTCTTTT	CAAGATGCCT	GAGGAAGTGC	100
14201.13	51	-----	-----	-----	-----	-----	100
		110	120	130	140	150	
Hsp 90	101	ACCATGGAGA	GGAGGAGGTG	GAGACTTTTG	CCTTTCAGGC	AGAAATTGCC	150
14201	101	-----	-----	-----	-----	-----	150
14201.3	101	ACCATGGAGA	GGAGGAGGTG	GAGACTTTTG	CCTTTCAGGC	AGAAATTGCC	150
14201.5	101	ACCATGGAGA	GGAGGAGGTG	GAGACTTTTG	CCTTTCAGGC	AGAAATTGCC	150
14201.13	101	-----	-----	-----	-----	-----	150
		160	170	180	190	200	
Hsp 90	151	CAACTCATGT	CCCTCATCAT	CAATACCTTC	TATTCCAACA	AGGAGATTTT	200
14201	151	-----	-----	-----	-----	-----	200
14201.3	151	CAACTCATGT	CCCTCATCAT	CAATACCTCC	TATTCCAACA	AGGAGATTNT	200
14201.5	151	CAACTCATGT	CCCTCATCAT	CAATACCTCC	TATTCCAACA	AGGAGATTTT	200
14201.13	151	-----	-----	-----	-----	-----	200
		210	220	230	240	250	
Hsp 90	201	CCTTCGGGAG	TTGATCTCTA	ATGCTTCTGA	TGCCTTGGAC	AAGATTCGCT	250
14201	201	-----	-----	-----	-----	-----	250
14201.3	201	CCTTCGGGAG	TTGATCTCTA	ATGCTTCTGA	TGCCTCGGAC	AAGATTCGCT	250
14201.5	201	CCTTCGGGAG	TTGATCTCTA	ATGCTTCTGA	TGCCTTGGAC	AAGATTCGCT	250
14201.13	201	-----	-----	-----	-----	-----	250
		260	270	280	290	300	
Hsp 90	251	ATGAGAGCCT	GACAGACCCT	TCGAAGTTGG	ACAGTGGTAA	AGAGCTGAAA	300
14201	251	-----	-----	-----	-----	-----	300
14201.3	251	ATGANAGCCT	GACAGACCCT	TCGAAGTNGG	TCAGCGGCAA	NGAGCTGAAA	300
14201.5	251	ATGAGAGCCT	GACAGACCCT	TCGAAGTTGG	ACAGTGGTAA	AGAGCTGAAA	300
14201.13	251	-----	-----	-----	-----	-----	300

FIGURE 7A

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	310	320	330	340	350	
Hsp 90	301	ATTGACATCA	TCCCCAACCC	TCAGGAACGT	ACCCTGACTT	TGGTAGACAC 350
14201	301	-----	-----	-----	-----	350
14201.3	301	ATTGACATCA	TCCCCAACCC	TCAGGAACGT	NCCCTGACTT	TGGTAGACAC 350
14201.5	301	ATTGACATCA	TCCCCAACCC	TCAGGAACGT	ACCCTGACTT	TGGTAGACAC 350
14201.13	301	-----	-----	-----	-----	350
	360	370	380	390	400	
Hsp 90	351	AGGCATTGGC	ATGACCAAAG	CTGATCTCAT	AAaTAATTtG	GGAACCATTG 400
14201	351	-----	-----	-----	-----	400
14201.3	351	AGGCATTGGC	ATGAaacAAG	CTGacCTCAT	NAnTTATTcG	GGgAaCcaTt 400
14201.5	351	AGGCATcGGC	ATGACCAAAG	CTGATCTCAT	AAaTAATTnG	GGAACCATTG 400
14201.13	351	-----	-----	-----	-----	400
	410	420	430	440	450	
Hsp 90	401	CCAAGTCTGG	TACTAAAGCA	TTCATGGAGG	CTCTTCAGGC	TGGTGCAGAC 450
14201	401	-----	-----	-----	-----	450
14201.3	401	CCAAGTCTTG	TNCTAAAGCA	TTCATGGAGG	CTCTNCAGGN	TGGcGCAGAC 450
14201.5	401	NCAAGTCTGG	TACTAAAGCA	TTCATGGAGG	CTCTTCAGGC	TGGTGCAGAC 450
14201.13	401	-----	-----	-----	-----	450
	460	470	480	490	500	
Hsp 90	451	ATCTCCATGA	TTGGGCAGTT	tGGTGTGGC	TtTtATTCTG	CCTACTTGGT 500
14201	451	-----	-----	-----	-----	500
14201.3	451	ATCTCCANGA	TTNGGCAGNT	GGGTGTGGC	TTnTATTCTG	CCcACTTGGT 500
14201.5	451	ATCTCCATGA	TTGGGCAGTT	GGGTGTGNC	TTnTATTCTG	CCTcCTTGGT 500
14201.13	451	-----	-----	-----	-----	500
	510	520	530	540	550	
Hsp 90	501	GGCAGAGAAA	GTGGTTGTGA	TCAGAAAGCA	CAACGATGAT	GAacAGTATG 550
14201	501	-----	-----	-----	-----	550
14201.3	501	GGCAGAGAAA	NNT.....	550
14201.5	501	GGCAGAGAAA	GTNGTTGTGA	TCA.....	550
14201.13	501	-----	-----	-----	-----TT	GAgTAgTATG 550
	560	570	580	590	600	
Hsp 90	551	cTtgGgAGTc	TtCTGcTGGA	GGTTCCTTCA	CTgtGCGTGC	TGACcATGGT 600
14201	551	-----	-----	-----	-----	600
14201.3	551	600
14201.5	551	600
14201.13	551	-TcnGnAGT-	TaCTGnTGGA	GGTTCCTTCA	CTnnGCGTGC	TGAC-ATGGT 600
	610	620	630	640	650	
Hsp 90	601	GAGCCCATtG	GcAtgGGTAC	CAaAGTGATC	CTCCATCTtA	AAGAAGATCA 650
14201	601	-----	-----	-----	-----	650
14201.3	601	650
14201.5	601	650
14201.13	601	GAGCCCATnG	GgAggGGTAC	CAnAGTGATC	CTCCATCTcA	AAGAAGATCA 650

FIGURE 7B

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		660	670	680	690	700	
Hsp 90	651	GACAGAGTAC	CTAGAAgAGA	GGCGGgTCAA	AGaAGTAGTG	AaGAaGCATT	700
14201	651	-----	-----	-----	-----	-----	700
14201.3	651	700
14201.5	651	700
14201.13	651	GACAGAGTAC	CTAGAnGAGA	GGCGGatCAA	AGnAGTAGTG	AtGAnGCATc	700
		710	720	730	740	750	
Hsp 90	701	CTCAGtTCAT	AGGCTATCCC	ATCACCCTTT	aTTTGAGAA	GGaACGAGAG	750
14201	701	-----	-----	-----	-----	-----	750
14201.3	701	750
14201.5	701	750
14201.13	701	CTCAGaTCAT	AGGCTATCCC	ATCACCCTTT	nTTTGAGAA	GGnACGAGAG	750
		760	770	780	790	800	
Hsp 90	751	AAGGAaATTA	GtGATGATGA	GGCAGAGGAA	GAGAAaGGTG	AGAAaGAAGA	800
14201	751	-----	-----	-----	-----	-----	800
14201.3	751	800
14201.5	751	800
14201.13	751	AAGGAnATTA	GnGATGATGA	GGCAGAGGAA	GAGAAtGGTG	AGAAtGAAGA	800
		810	820	830	840	850	
Hsp 90	801	GGAAgATAAa	GATGATGAAG	AAAagCCCAA	GATCGAaGAT	GTGGgTTCAG	850
14201	801	-----	-----	-----	-----	-----	850
14201.3	801	850
14201.5	801	850
14201.13	801	GGAnGnTAaC	GATGATGAAG	AAAnCccCAA	GATCGatGAT	GTGGnTTCAG	850
		860	870	880	890	900	
Hsp 90	851	ATGAGGaGGA	TGACAGCGGT	aAgGATAAGA	AGAAGAAaAC	TAaGAagATC	900
14201	851	-----	-----	-----	-----	-----	900
14201.3	851	900
14201.5	851	900
14201.13	851	ATGAGGnGGA	TGACAGCGGT	nAnGATAAGA	AGAAGAAAnAC	TAnGAnnATC	900
		910	920	930	940	950	
Hsp 90	901	AAAGAGAAAT	ACATTGATCA	GGAAGAACTA	AACAAGACCA	AGCCTATTTG	950
14201	901	-----	-----	-----	-----	-----	950
14201.3	901	950
14201.5	901	950
14201.13	901	950
		960	970	980	990	1000	
Hsp 90	951	GACCAGAAAC	CCTGATGACA	TCACCCAAGA	GGAGTATGGA	GAATTCTACA	1000
14201	951	-----	-----	-----	-----	-----	1000
14201.3	951	1000
14201.5	951	1000
14201.13	951	1000

FIGURE 7C

10/20

Hsp 90	1001	1010	1020	1030	1040	1050	
14201	1001	AGAGCCTCAC	TAATGACTGG	GAAGACCACT	TGGCAGTCAA	GCACTTTTCT	1050
14201.3	1001	1050
14201.5	1001	1050
14201.13	1001	1050
Hsp 90	1051	1060	1070	1080	1090	1100	
14201	1051	GTAGAAGGTC	AGTTGGAATT	CAGGGCATTG	CTATTTATTC	CTCGTCGGGC	1100
14201.3	1051	1100
14201.5	1051	1100
14201.13	1051	1100
Hsp 90	1101	1110	1120	1130	1140	1150	
14201	1101	TCCCTTTGAC	CTTTTGTAGA	ACAAGAAGAA	AAAGAACAAC	ATCAAACCTCT	1150
14201.3	1101	-----AAGAA	AAAGAACAAC	ATCAAACCTCT	1150
14201.5	1101	1150
14201.13	1101	1150
Hsp 90	1151	1160	1170	1180	1190	1200	
14201	1151	ATGTCCGCCG	TGTGTTTCATC	ATGGaCAGCT	GTGATGAGTT	GATACCAGAG	1200
14201.3	1151	ATGTCCGCCG	TGTGTTTCATC	ATGGnCAGCT	GTGATGAGTT	GATACCAGAG	1200
14201.5	1151	1200
14201.13	1151	1200
Hsp 90	1201	1210	1220	1230	1240	1250	
14201	1201	TATCTCAATT	TTATCCGTGG	TGTGGTTGAC	TcTGAGGaTC	TGCCCCTGAA	1250
14201.3	1201	TATCTCAATT	TTATCCGTGG	TGTGGTTGAC	TnTGAGGnTC	TGCCCCTGAA	1250
14201.5	1201	1250
14201.13	1201	1250
Hsp 90	1251	1260	1270	1280	1290	1300	
14201	1251	CATCTCCCGa	GAAATGCTCC	AGCAGAGCAA	AATCTTGAAA	GtCATTTCGCA	1300
14201.3	1251	CATCTCCCGn	GAAATGCTCC	AGCAGAGCAA	AATCTTGAAA	GgCATTTCGCA	1300
14201.5	1251	1300
14201.13	1251	1300
Hsp 90	1301	1310	1320	1330	1340	1350	
14201	1301	AAAACATTGT	TAAGaAGTGC	CTTgAGCTCT	TCTCTgAGCT	GGCAGAAGaC	1350
14201.3	1301	AAAACATTGT	TAAGnAGTGC	CTTnAGCTCT	TCTCTnAGCT	GGCAGAAGnC	1350
14201.5	1301	1350
14201.13	1301	1350

FIGURE 7D

11/20

		1360	1370	1380	1390	1400	
Hsp 90	1351	AAGGAGAATT	ACAAGAAATT	CTATGAGGCA	TTCTCTAAAA	ATCTCAAGCT	1400
14201	1351	AAGG-GGATT	TCAAGAAATT	CTTTGGGG--	-----	-----	1400
14201.3	1351	1400
14201.5	1351	1400
14201.13	1351	1400
		1410	1420	1430	1440	1450	
Hsp 90	1401	TGGAATCCAC	GAAGACTCCA	CTAACCGCCG	CCGCCTGTCT	GAGCTGCTGC	1450
14201	1401	-----	-----	-----	-----	-----	1450
14201.3	1401	1450
14201.5	1401	1450
14201.13	1401	1450
		1460	1470	1480	1490	1500	
Hsp 90	1451	GCTATCATAC	CTCCCAGTCT	GGAGATGAGA	TGACATCTCT	GTCAGAGTAT	1500
14201	1451	-----	-----	-----	-----	-----	1500
14201.3	1451	1500
14201.5	1451	1500
14201.13	1451	1500
		1510	1520	1530	1540	1550	
Hsp 90	1501	GTTTCTCGCA	TGAAGGAGAC	ACAGAAAGTCC	ATCTATTACA	TCACTGGTGA	1550
14201	1501	-----	-----	-----	-----	-----	1550
14201.3	1501	1550
14201.5	1501	1550
14201.13	1501	1550
		1560	1570	1580	1590	1600	
Hsp 90	1551	GAGCAAAGAG	CAGGTGGCCA	ACTCAGCTTT	TGTGGAGCGA	GTGCGGAAAC	1600
14201	1551	-----	-----	-----	-----	-----	1600
14201.3	1551	1600
14201.5	1551	1600
14201.13	1551	1600
		1610	1620	1630	1640	1650	
Hsp 90	1601	GGGGCTTCGA	GGTGGTATAT	ATGACCGAGC	CCATTGACGA	GTA CTGTGTG	1650
14201	1601	-----	-----	-----	-----	-----	1650
14201.3	1601	1650
14201.5	1601	1650
14201.13	1601	1650

FIGURE 7E

12/20

		1660	1670	1680	1690	1700	
Hsp 90	1651	CAGCAGCTCA	AGGAATTGTA	TGGGAAGAGC	CTGGTCTCAG	TTACCAAGGA	1700
14201	1651	-----	-----	-----	-----	-----	1700
14201.3	1651	1700
14201.5	1651	1700
14201.13	1651	1700
		1710	1720	1730	1740	1750	
Hsp 90	1701	GGGTCTGGAG	CTGCCTGAGG	ATGAGGAGGA	GAAGAAGAAG	ATGGAAGAGA	1750
14201	1701	-----	-----	-----	-----	-----	1750
14201.3	1701	1750
14201.5	1701	1750
14201.13	1701	1750
		1760	1770	1780	1790	1800	
Hsp 90	1751	GCAAGGCAAA	GTTTGAGAAC	CTCTGCAAGC	TCATGAAAGA	AATCTTAGAT	1800
14201	1751	1800
14201.3	1751	1800
14201.5	1751	1800
14201.13	1751	1800
		1810	1820	1830	1840	1850	
Hsp 90	1801	AAGAAGGTG	AGAAGGTGAC	AATCTCCAAT	AGACTTGTGT	CTTCACCTTG	1850
14201	1801	1850
14201.3	1801	1850
14201.5	1801	1850
14201.13	1801	1850
		1860	1870	1880	1890	1900	
Hsp 90	1851	CTGCATTGTG	ACCAGCACCT	ACGGCTGGAC	AGCCAATATG	GAGCGGATCA	1900
14201	1851	1900
14201.3	1851	1900
14201.5	1851	1900
14201.13	1851	1900
		1910	1920	1930	1940	1950	
Hsp 90	1901	TGAAAGCCCA	GGCACTTCGG	GACAACTCCA	CCATGGGCTA	TATGATGGCC	1950
14201	1901	1950
14201.3	1901	1950
14201.5	1901	1950
14201.13	1901	1950
		1960	1970	1980	1990	2000	
Hsp 90	1951	AAAAAGCACC	TGGAGATCAA	CCCTGACCAC	CCCATTGTGG	AGACGCTGCG	2000
14201	1951	2000
14201.3	1951	2000
14201.5	1951	2000
14201.13	1951	2000

FIGURE 7F

13/20

		2010	2020	2030	2040	2050	
Hsp 90	2001	GCAGAAGGCT	GAGGCCGACA	AGAATGATAA	GGCAGTTAAG	GACCTGGTGG	2050
14201	2001	2050
14201.3	2001	2050
14201.5	2001	2050
14201.13	2001	2050
		2060	2070	2080	2090	2100	
Hsp 90	2051	TGCTGCTGTT	TGAAACCGCC	CTGCTATCTT	CTGGCTTTTC	CCTTGAGGAT	2100
14201	2051	2100
14201.3	2051	2100
14201.5	2051	2100
14201.13	2051	2100
		2110	2120	2130	2140	2150	
Hsp 90	2101	CCCCAGACCC	ACTCCAACCG	CATCTATCGC	ATGATCAAGC	TAGGTCTAGG	2150
14201	2101	2150
14201.3	2101	2150
14201.5	2101	2150
14201.13	2101	2150
		2160	2170	2180	2190	2200	
Hsp 90	2151	TATTGATGAA	GATGAAGTGG	CAGCAGAGGA	ACCCAATGCT	GCAGTTCCTG	2200
14201	2151	2200
14201.3	2151	2200
14201.5	2151	2200
14201.13	2151	2200
		2210	2220	2230	2240	2250	
Hsp 90	2201	ATGAGATCCC	CCCTCTCGAG	GGCGATGAGG	ATGCCGTCTCG	CATGGAAGAA	2250
14201	2201	2250
14201.3	2201	2250
14201.5	2201	2250
14201.13	2201	2250
		2260	2270	2280	2290	2300	
Hsp 90	2251	GTCGATTAGG	TTAGGAGTTC	ATAGTTGGAA	AACTTG TGCC	CTTGATATAGT	2300
14201	2251	2300
14201.3	2251	2300
14201.5	2251	2300
14201.13	2251	2300
		2310	2320	2330	2340	2350	
Hsp 90	2301	GTCCCCATGG	GCTCCCACTG	CAGCCTCGAG	TGCCCTGTC	CCACCTGGCT	2350
14201	2301	2350
14201.3	2301	2350
14201.5	2301	2350
14201.13	2301	2350

FIGURE 7G

14/20

		2360	2370	2380	2390	2400	
Hsp 90	2351	CCCCCTGCTG	GTGTCTAGTG	TTTTTTTCCC	TCTCCTGTCC	TTGTGTTGAA	2400
14201	2351	2400
14201.3	2351	2400
14201.5	2351	2400
14201.13	2351	2400
		2410	2420	2430	2440	2450	
Hsp 90	2401	GGCAGTAAAC	TAAGGGTGTC	AAGCCCCATT	CCCTCTCTAC	TCTTGACAGC	2450
14201	2401	2450
14201.3	2401	2450
14201.5	2401	2450
14201.13	2401	2450
		2460	2470	2480	2490	2500	
Hsp 90	2451	AGGATTGGAT	GTTGTGTATT	GTGGTTTATT	TTATTTTCTT	CATTTTGTTT	2500
14201	2451	2500
14201.3	2451	2500
14201.5	2451	2500
14201.13	2451	2500
		2510	2520	2530	2540	2550	
Hsp 90	2501	TGAAATTAAA	GTATGCAAAA	TAAAGAATAT	GCCGTTTTTA	TAC.....	2550
14201	2501	2550
14201.3	2501	2550
14201.5	2501	2550
14201.13	2501	2550

FIGURE 7H

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		10	20	30	40	50	
capthepsin	1	TCCGGCAACG	CCAACCGCTC	CGCTGCGCGC	AGGCTGGGCT	GCAGGCTCTC	50
87058	1	-----	-----	-----	-----	-----	50
87058.6	1	-----	-----	-----	-----	-----	50
87058.8	1	-----	-----	-----	-----	-----	50
87058.16	1	-----	-----	-----	-----	-----	50
		60	70	80	90	100	
capthepsin	51	GGCTGCAGCG	CTGGGCTGGT	GTGCAGTGGT	GCGACCACGG	CTCACGGCAG	100
87058	51	-----	-----	-----	-----	-----	100
87058.6	51	-----	-----	-----	-----	-----	100
87058.8	51	-----	-----	-----	-----	-----	100
87058.16	51	-----NCN	GGTTGAGNAT	TCCGACNAGT	CCGAAAACGT	CCGGCAAGTC	100
		110	120	130	140	150	
capthepsin	101	CCTCAGCCAC	CCAGATGTAA	GCGATCTGGT	TCCCACCTCA	GCCTCCCAG	150
87058	101	-----	-----	-----	-----	-----	150
87058.6	101	-----	-----	-----	-----	-----	150
87058.8	101	-----	-----	-----	-----	-----	150
87058.16	101	ACCCGCTCCG	CTGNCGCAG	GCTGGGNTGC	AGGCTCTCGG	NTGCAGNGCT	150
		160	170	180	190	200	
capthepsin	151	TAGTGGATCT	AGGATCCGGC	TTCCAACATG	TGGCAGtTCT	GGGCCTCCCT	200
87058	151	-----	-----	-----	-----	-----	200
87058.6	151	-----	-----	-----	-----	-----	200
87058.8	151	-----	-----	-----	-----	-----	200
87058.16	151	GGGTGGATCT	AGGATCCGGC	TTCCAACATG	TGGCAGtTCT	GGGCCTCCCT	200
		210	220	230	240	250	
capthepsin	201	CTGcTGCCTG	CTGGTGTGG	cCAATGCCCG	GAGcAGGcCC	TCTTTCCATC	250
87058	201	-----	-----	-----	-----	-----	250
87058.6	201	-----	-----	-----	-----	-----	250
87058.8	201	-----	-----	-----	-----	-----	250
87058.16	201	CTGnTGCCTG	CTGGTGTGG	aCAATGCCCG	GAGgAGGnCC	TCTTTCCATC	250
		260	270	280	290	300	
capthepsin	251	CCCTGTGCGA	TGAGCTGGTC	AaCTATGTCA	ACAAACGGAA	TACCACGTGG	300
87058	251	-----	-----	-----	-----	-----	300
87058.6	251	-----	-----	-----	-----	-----	300
87058.8	251	-----	-----	-----	-----	-----	300
87058.16	251	CCCTGTGCGA	TGAGCTGGTC	AnCTATGTCA	ACAAACGGAA	TACCACGTGG	300

FIGURE 8A

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		310	320	330	340	350	
capthepsin	301	cAGGCCGgAa	ACAACtTCTA	CAACGTGGAC	ATGAGCTACT	TGAaGAGGcT	350
87058	301	-----	-----	-----	-----	-----	350
87058.6	301	-----	-----	-----	-----	-----	350
87058.8	301	-----	-----	-----	-----	-----	350
87058.16	301	nAGGCCGgGa	ACAACtTCTA	CAACGTGGAC	ATGAGCTACT	TGAaGAGGnT	350
		360	370	380	390	400	
capthepsin	351	ATGTGGTACC	TTCCTGGGTG	GGCCCAAGCC	ACCCAGAGA	GTTATGTTTA	400
87058	351	-----	-----	-----	-----	-----	400
87058.6	351	-----	-----	-----	-----	-----	400
87058.8	351	--GaGGTACC	TTCCTGGGTG	GGCCCAAGCC	ACCCAGAGA	GTTATGTTTA	400
87058.16	351	ATGTGGTACC	TTCCTGGGTG	GGCCCAAGCC	ACCCAGAGA	GTTnTGTTTA	400
		410	420	430	440	450	
capthepsin	401	CCGAGGACCT	GAAGCTGCCT	GCAAGCTTCG	ATGCACGGGA	ACAATGGCCA	450
87058	401	-----	-----	-----	-----	-----	450
87058.6	401	-----	-----	-----	-----	-----	450
87058.8	401	CCGAGGACCT	GAAGCTGCCT	GCAAGCTTCG	ATGCACGGGA	ACAATGGCCA	450
87058.16	401	CCGAGGACCT	GANGCTGCCT	GCAAGCTTCG	AaGgACGGGA	ACAATGGCCA	450
		460	470	480	490	500	
capthepsin	451	CAGTGtCCCA	CCATCAAAGA	GATCAGAGAC	CAGGGCTCCT	GTGGCTCCTG	500
87058	451	-----	-----	-----	-----	-----	500
87058.6	451	-----	-----	-----	-----	-----	500
87058.8	451	CAGTGtCCCA	CCATCAAAGA	GATCAGAGAC	CAGGGnTCCT	GTGGCTCCTG	500
87058.16	451	CAGTGtCCCA	CCATCAAAGA	GATCAGAGAN	CAGGGCTCCT	GTGGnTCCTG	500
		510	520	530	540	550	
capthepsin	501	CTGGGCCTTC	GGGGCTGTGG	AAGCCATCTC	TGACCGGATC	TGCATCCACA	550
87058	501	-----	-----	-----	-----	-----	550
87058.6	501	-----	-----	-----	-----	-----	550
87058.8	501	CTGGGCCTTC	GGGGCTGTGG	AAGCCATCTC	TGACCGGATC	TGNATCCACA	550
87058.16	501	CTGGGCCTcC	GGGGCTGTGG	AAGNCATCTC	TGACCGGATC	TGCATCCACA	550
		560	570	580	590	600	
capthepsin	551	CCAATGCGCA	CGTCAGCGTG	GAGGTGTGCG	CGGAGGACCT	GCTCACATGC	600
87058	551	-----	-----	-----	-----	-----	600
87058.6	551	-----	-----	-----	-----	-----	600
87058.8	551	CCAATGCGCA	CGTCAGCGTG	GAGGTGTGCG	CGGAGGAC-T	GCTCACATGC	600
87058.16	551	CCAATGNGCA	CGTCAGCGTG	GtGGTGTGCG	NGGAGGACCT	GaTCACCTnT	600
		610	620	630	640	650	
capthepsin	601	TGTGGCAGCA	TGTGTGGGGA	CGGCTGTAAT	GGTGGCTATC	CTGCTGAAGC	650
87058	601	-----	-----	-----	-----	-----	650
87058.6	601	-----	-----	-----	-----	---gTGAAGC	650
87058.8	601	TGTGGCAGNA	TGTGTGGGGA	CGGCTGTAAT	GGTGGCTATC	CTGCTGAAGC	650
87058.16	601	TGTGGtAGCA	TGTGTGGGGA	CGGCTGTAAT	GGTGGtTATC	CTGnTGAAGC	650

FIGURE 8B

17/20

		660	670	680	690	700	
capthepsin	651	TTGGA	ACTTC	TGGACA	AAGAA	AAGGCCTGGT	TTCTGGTGGC
87058	651	-----	-----	-----	-----	-----	-----
87058.6	651	TTGGA	ACTTC	TGGACA	AAGAA	AAGGCCTGGT	TTCTGGTGGC
87058.8	651	TTGGA	ACTTC	TGGACA	AAGAA	AAGGCCTGGT	TTCTGGTGGC
87058.16	651	TNGGg	NCTTC	TNaga	AAGAA	AAGGCtNGtT	TT--GGTGGC
		710	720	730	740	750	
capthepsin	701	CCCATG	TAGG	GTGCAG	ACCG	TACTCCATCC	CTCCCTGTGA
87058	701	-----	-----	-----	-----	-----	-----
87058.6	701	CCCATG	TAGG	GTGCAG	ACCG	TACTCCATCC	CTCCCTGTGA
87058.8	701	CCCATG	TAGG	GTGTAG	ACCG	TACTCCATCC	CTCCCTGTGA
87058.16	701	CCCATGT
		760	770	780	790	800	
capthepsin	751	AACGG	GCTCCC	GGCCCC	CATG	CACGGGGGAG	GGAGATACCC
87058	751	-----	-----	-----	-----	-----	-----
87058.6	751	AACGG	GCTCCC	GGCCCC	CATG	CACGGGGGAG	GGAGATACCC
87058.8	751	AACGG	GtTCCC	GGgCCCC	CATG	CACGGNGGAG	GGAGATACCC
87058.16	751
		810	820	830	840	850	
capthepsin	801	CAAGAT	CTGT	GAGCCT	TGGCT	ACAGCCCGAC	CTACAAACAG
87058	801	-----	-----	-----	-----	-----	-----
87058.6	801	CAAGAT	CTGT	GAGCCT	TGGCT	ACAGCCCGAC	CTACAAACAG
87058.8	801	CAAGAT	CTGT	GAGCCT	TGGgT	ACAGtCCcga	CcACAAACAG
87058.16	801
		860	870	880	890	900	
capthepsin	851	ACGGATA	CAA	TTCCTA	CAGC	GTCTCCAATA	GCGAGAAGGA
87058	851	-----	-----	-----	-----	-----	-----
87058.6	851	ACGGATA	CAA	TTCCTA	CAGC	GTCTCCAATA	GCGAGAAGGA
87058.8	851	ACGGATA	CAA	TTCCT-	CAGN	GTCTCCAATA	GtGAGAAGGA
87058.16	851
		910	920	930	940	950	
capthepsin	901	GAGAT	CTACA	AAAAC	GGCCC	CGTGGAGGGA	GCTTTCTCTG
87058	901	-----	-----	-----	-----	-----	-----
87058.6	901	GAGAT	CTACA	AAAAC	GGCCC	CGTGGAGGGA	GCTTTCTCTG
87058.8	901	GAGAT	CTACA	AtAAC	GGC..
87058.16	901
		960	970	980	990	1000	
capthepsin	951	CTTCCT	GCTC	TACAAG	TCAG	GAGTGTACCA	ACACGTCACC
87058	951	-----	-----	-----	-----	-----	-----
87058.6	951	CTTCCT	GCTC	TACAAG	TCAG	GAGTGTACCA	ACACGTCACC
87058.8	951
87058.16	951

FIGURE 8C

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		1010	1020	1030	1040	1050	
capthepsin	1001	TGGGTGGCCA	TGCCATCCGC	ATCCTGGGCT	GGGGAGTGGA	GAATGGCACA	1050
87058	1001	-----cGg	cagacGCCAA	CTCCTGGAAC	ACTGACTGGG	GTGACAATGG	1050
87058.6	1001	TGGGTGGCCA	TGCCATCCGC	ATCCTGGGCT	GGGGAGTGGA	GAATGGCACA	1050
87058.8	1001	1050
87058.16	1001	1050
		1060	1070	1080	1090	1100	
capthepsin	1051	cCCTACTGGC	TGGTTGCCAA	CTCCTGGAAC	ACTGACTGGG	GTGACAATGG	1100
87058	1051	-----cGg	cagacGCCAA	CTCCTGGAAC	ACTGACTGGG	GTGACAATGG	1100
87058.6	1051	aCCTACTGGC	TGGTTGgCAA	CTCCTGGAAC	ACTGACTGGG	GTGACAATGG	1100
87058.8	1051	1100
87058.16	1051	1100
		1110	1120	1130	1140	1150	
capthepsin	1101	CTTCTTTAAA	ATACTCAGAG	GACAGGATCA	CTGTGGAATC	GAATCAGAAG	1150
87058	1101	CTTCTTTAAA	ATACTCAGAG	GACAGGTTCA	CTGTGGAATC	GAATCAGAAG	1150
87058.6	1101	gTTC-----	-----	-----	-----	-----	1150
87058.8	1101	1150
87058.16	1101	1150
		1160	1170	1180	1190	1200	
capthepsin	1151	TGGTGGCTGG	AATTCCACGC	ACCGATCAGT	ACTGGGAAAA	GATCTAATCT	1200
87058	1151	TGGTGGCTGG	AATTCCACGC	ACCGTTCAGT	ACTGGGAAAA	GNTCTAATCT	1200
87058.6	1151	-----	-----	-----	-----	-----	1200
87058.8	1151	1200
87058.16	1151	1200
		1210	1220	1230	1240	1250	
capthepsin	1201	GCCGTGGGCC	TGTCGTGCCA	GTCCTGGGGG	CGAGATCGGG	GTAGAAATGC	1250
87058	1201	GCCGTGGGCC	TNTCGTGCCA	GTCCTGGGGG	CGAGATCGGG	GTAGAAATGC	1250
87058.6	1201	-----	-----	-----	-----	-----	1250
87058.8	1201	1250
87058.16	1201	1250
		1260	1270	1280	1290	1300	
capthepsin	1251	ATTTTATTCT	TTAAGTTCAC	GTAAGATACA	AGTTTCAGgC	AGGGTCTgAA	1300
87058	1251	ATTTTATTCT	TTAAGTTCAC	GTAAGATACA	AGTTTCAGaC	AGGGTCTnAA	1300
87058.6	1251	1300
87058.8	1251	1300
87058.16	1251	1300
		1310	1320	1330	1340	1350	
capthepsin	1301	GGaCTGGaTT	gGCCAAAcAT	CAGACCTGTC	TTCCAAGGAG	ACCAAGTCCT	1350
87058	1301	GGcCTGGnTT	ngCCAAAnAT	CAGACCTGT.	1350
87058.6	1301	1350
87058.8	1301	1350
87058.16	1301	1350

FIGURE 8D

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		1360	1370	1380	1390	1400	
capthepsin	1351	GGCTACATCC	CAGCCTGTGG	TTACAGTGCA	GACAGGCCAT	GTGAGCCACC	1400
87058	1351	1400
87058.6	1351	1400
87058.8	1351	1400
87058.16	1351	1400
		1410	1420	1430	1440	1450	
capthepsin	1401	GCTGCCAGCA	CAGAGCGTCC	TTCCCCCTGT	AGACTAGTGC	CGTGGGAGTA	1450
87058	1401	1450
87058.6	1401	1450
87058.8	1401	1450
87058.16	1401	1450
		1460	1470	1480	1490	1500	
capthepsin	1451	CCTGCTGCCC	AGCTGCTGTG	GCCCCCTCCG	TGATCCATCC	ATCTCCAGGG	1500
87058	1451	1500
87058.6	1451	1500
87058.8	1451	1500
87058.16	1451	1500
		1510	1520	1530	1540	1550	
capthepsin	1501	AGCAAGACAG	AGACGCAGGA	TGGAAGCGG	AGTTCCTAAC	AGGATGAAAG	1550
87058	1501	1550
87058.6	1501	1550
87058.8	1501	1550
87058.16	1501	1550
		1560	1570	1580	1590	1600	
capthepsin	1551	TTCCCCCATC	AGTTCCCCCA	GTACCTCCAA	GCAAGTAGCT	TTCCACATTT	1600
87058	1551	1600
87058.6	1551	1600
87058.8	1551	1600
87058.16	1551	1600
		1610	1620	1630	1640	1650	
capthepsin	1601	GTCACAGAAA	TCAGAGGAGA	GATGGTGTG	GGAGCCCTTT	GGAGAACGCC	1650
87058	1601	1650
87058.6	1601	1650
87058.8	1601	1650
87058.16	1601	1650

FIGURE 8E

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		1660	1670	1680	1690	1700	
capthepsin	1651	AGTCTCCAGG	TCCCCCTGCA	TCTATCGAGT	TTGCAATGTC	ACAACCTCTC	1700
87058	1651	1700
87058.6	1651	1700
87058.8	1651	1700
87058.16	1651	1700
		1710	1720	1730	1740	1750	
capthepsin	1701	TGATCTTG TG	CTCAGCATGA	TTCTTTAATA	GAAGTTTAT	TTTTCGTGCA	1750
87058	1701	1750
87058.6	1701	1750
87058.8	1701	1750
87058.16	1701	1750
		1760	1770	1780	1790	1800	
capthepsin	1751	CTCTGCTAAT	CATGTGGGTG	AGCCAGTGGA	ACAGCGGGAG	CCTGTGCTGG	1800
87058	1751	1800
87058.6	1751	1800
87058.8	1751	1800
87058.16	1751	1800
		1810	1820	1830	1840	1850	
capthepsin	1801	TTTGCAAGATT	GCCTCCTAAT	GACGCGGCTC	AAAAGGAAAC	CAAGTGGTCA	1850
87058	1801	1850
87058.6	1801	1850
87058.8	1801	1850
87058.16	1801	1850
		1860	1870	1880	1890	1900	
capthepsin	1851	GGAGTTGTTT	CTGACCCACT	GATCTCTACT	ACCACAAGGA	AAATAGTTTA	1900
87058	1851	1900
87058.6	1851	1900
87058.8	1851	1900
87058.16	1851	1900
		1910	1920	1930	1940	1950	
capthepsin	1901	GGAGAAACCA	GCTTTTACTG	TTTTTGAAAA	ATTACAGCTT	CACCCTGTCA	1950
87058	1901	1950
87058.6	1901	1950
87058.8	1901	1950
87058.16	1901	1950
		1960	1970	1980	1990	2000	
capthepsin	1951	AGTTAACAAG	GAATGCCTGT	GCCAATAAAA	GGTTTCTCCA	ACTTGA....	2000
87058	1951	2000
87058.6	1951	2000
87058.8	1951	2000
87058.16	1951	2000

FIGURE 8F

INTERNATIONAL SEARCH REPORT

International Application No

PC./US 96/08501

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 6 C12Q1/68 C12P19/34 C12N15/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12Q C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PCR PROTOCOLS: A GUIDE TO METHODS AND APPLICATIONS. EDITOR INNIS M.; PUBLISHER ACADEMIC, 1990, SAN DIEGO, CALIF., pages 219-27, XP002015609 OCHMAN, H. ET AL: "Amplification of flanking sequences by inverse PCR" see whole article ---	1-8
X	BIOTECHNIQUES, vol. 18, no. 5, May 1995, pages 762-64, XP000509322 COOLIDGE C ET AL: "Run-around PCR: A novel way to create duplications using polymerase chain reaction " see the whole document --- -/--	1-8



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

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Date of the actual completion of the international search

10 October 1996

Date of mailing of the international search report

25.10.96

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Osborne, H

INTERNATIONAL SEARCH REPORT

International Application No

PC./US 96/08501

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF BIOLOGICAL CHEMISTRY., vol. 268, no. 12, 1993, pages 8842-50, XP000604943 LEE, D. ET AL.: "Molecular cloning and genomic organization of a gene for luciferin-binding protein from dinoflagellate Gonyaulax polyedra" see the whole document ---	1-8
X	US,A,4 994 370 (SILVER) 19 February 1991 see the whole document ---	1-8
X	JOURNAL OF VIROLOGICAL METHODS, vol. 49, no. 3, January 1994, pages 269-84, XP000606337 TSUEI D-J ET AL: "Inverse polymerase chain reaction for cloning cellular sequences adjacent to integrated hepatitis b virus in hepatocellular carcinomasw" see the whole document ---	1-8
X	WO,A,90 14423 (THE INFERGENE CO.) 29 November 1990 see page 19 ---	1-8
A	WO,A,93 12257 (HYBRITECH INC) 24 June 1993 see the whole document ---	1-8
A	NUCLEIC ACIDS RESEARCH, vol. 19, 1991, pages 3055-60, XP002015610 PARKER J. ET AL: "Walking PCR" cited in the application -----	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PC./US 96/08501

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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WO-A-9014423	29-11-90	NONE	

WO-A-9312257	24-06-93	AU-A- 3274793	19-07-93
		US-A- 5512463	30-04-96
